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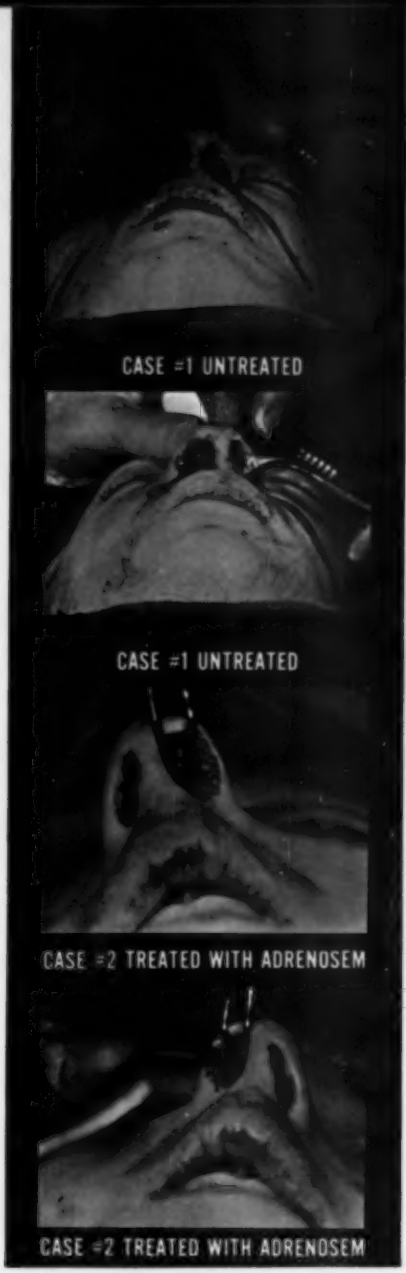

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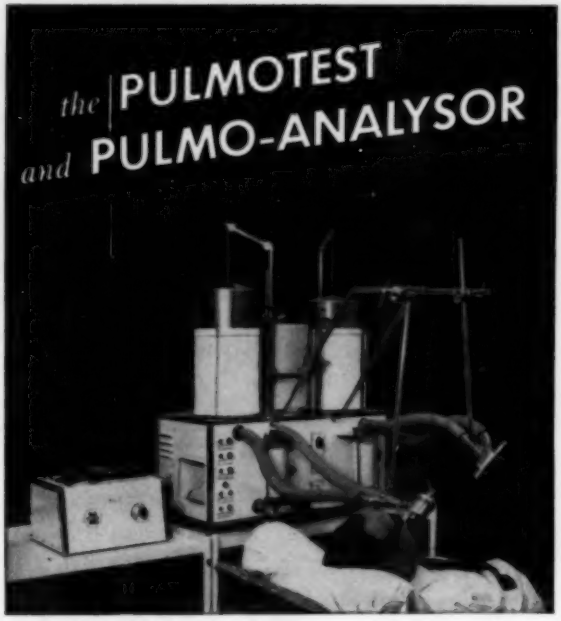

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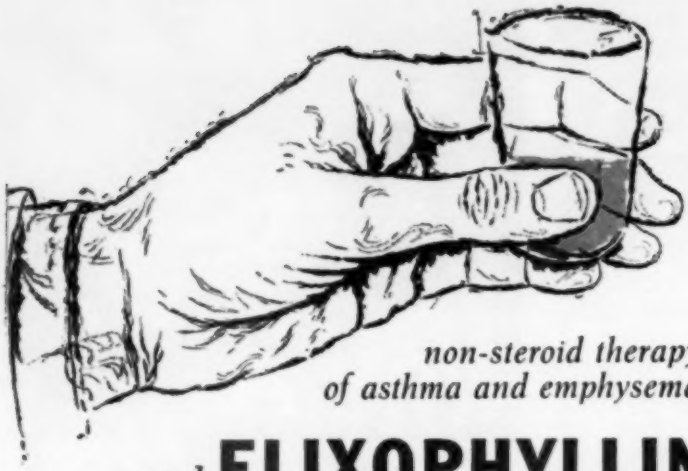

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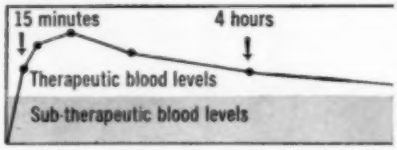

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THE AMERICAN REVIEW OF TUBERCULOSIS AND PULMONARY DISEASES

VOLUME 78 *August, 1958* NUMBER 2

THE ARCANA OF TUBERCULOSIS

With a Brief Epidemiologic History of the Disease in the U.S.A.

E. R. N. GRIGG[1]

(Received for publication January 6, 1958)

Tuberculosis comes and goes

ALFRED FLATZECK-HOFBAUER

Forty years ago, an hygienist "was forced to the conclusion that the real significance of the various factors concerned in tuberculosis can not be ascertained with the incomplete and superficial knowledge, relatively inaccurate methods, and the illogical reasoning hitherto so commonly brought to bear upon the question" (1). As of late, this pessimistic, or maybe just realistic, viewpoint was shared by a phthisiologist, who wrote: "At any meeting of physicians interested in tuberculosis . . . the casual observer must be impressed by the frequency with which confessions of ignorance are uttered. The plain truth is that the enigma of tuberculosis is far from being solved . . ." (2).

Could there be a relationship between the stalemate in the advancement of knowledge in tuberculology and the absence of original working hypotheses in this field? The rigid philosophic motivation of the classical germ theory, so resplendent and dignified in the 1890s, at the height of its rising glory, is now in many ways obsolete, not to mention jade-inflicted by excessive wear. With the utterly naive, and predominantly finalistic, approach of prevailing medical theoretics, many people conceive immunity as a successful warfare, waged by adequately numbered and properly trained soldiers (antibodies) against ruthless invaders (antigen). Such antiquated concepts and the absurd, but nonetheless widely accepted, dogma of the fixity of diseases must be repudiated (3) before one can hope to solve the acid-fast mystery. On the other hand, even if the enigma of tuberculosis were to remain unsolved, incontrovertible statistical observations have demonstrated that, from an epidemiologic viewpoint, tuberculosis is a cyclic and, as such, a self-limiting phenomenon.

I. INTRODUCTION

Is Tuberculosis a Contagious Disease?

The idea of contagion (by evil spirits) is as old as the pristine religious concepts, and has its roots in the primitive's animism and in the basic human trait which tends to ascribe extrinsic causalities to natural phenomena.

[1] Kankakee, Illinois.

The concept of the air-borne transmission of miasmatic or pestilential diseases (including phthisis), which is commonly credited to Aristotle (4), was accepted for almost two millenia, and can be retraced to the *protidio-koino-miasma* and *peridio-koino-miasma* of Edward Miller (1760–1812) of New York. The latter's etimologic aberrations, endorsed by the famous Rush of Philadelphia, were mentioned in connection with tuberculosis as late as 1878 in Cincinatti (5).

A seldom remembered contagionistic precursor in this country was the New England preacher and self-styled medical theorist John Walton (eighteenth century). In regard to consumption, he assumed a Sydenhamic "error in the non-naturals," and postulated the existence of *disagreeable Particles* of spheroidal, conical, or cubical shape ("neither pointed, nor large, as these would lead to Canker") (6). A more scientific approach can be found in the theory of "universal parasitism," proposed by John Crawford (1746–1813), professor in the Medical College of Baltimore. He believed that consumption is caused by a *contagium virum*, and cited a case of conjugal phthisis to prove his contention (7).

As it happened, Klencke (in 1843) and Erdt (in 1853) had demonstrated that tuberculosis can be transmitted, but Villemin received the priority credit, perhaps because he proved it beyond reasonable doubt. Likewise, Aufrecht (8) and von Baumgarten (9) stained and recognized *Tuberkelbakterien*, but the only one to achieve lasting fame was the *Bacillus* (rechristened later *Mycobacterium*) *tuberculosis* of the "very persuasive Robert Koch, who made it a habit to delete mention of his use of other men's methods, but seldom forgot to acknowledge their failures" (10).

There remains today no doubt that tuberculosis is a contagious disease in the sense of infectious illness, i.e., caused by a living antigen. However, in order to be complete, this statement must receive a few semantic qualifications.

In 1818, the ultradoct founder of the University of Maryland, Nathaniel Potter (1770-1843), made a "distinction between vitiated secretion, the result of living vascular action" (contagion), and "(the result) of putrefaction in dead matter" (infection). Today, the terms *infectious*, *communicable*, and *contagious* (as well as the colloquial *catching*) are largely synonymous. And yet, *infection* emphasizes the actual introduction of antigen into the afflicted body, *communicability* indicates potential transmission, and *contagiousness* stresses the hazard which results from direct or indirect contact. Pneumococcal pneumonia is certainly an infectious illness; yet, it can hardly be listed among the communicable diseases, even though it may be slightly contagious. The discrepancy stems from the assumed ubiquitousness of pneumococcus.

The classical germ theory had a corollary, the concept of universal susceptibility (11), which equated infection with accomplished contagion. Acceptance of this corollary contributed to the synonymy of infectious, communicable, and contagious. (It may be stated parenthetically that virulent strains of *M. tuberculosis* had been isolated from the nasal cavities of asymptomatic contacts (12). Similar occurrences, observed in the course of infections with other microorganisms, were explained by using the contradictory terminology of "subclinical disease." In the case of tuberculosis, such difficulties were overcome by assuming the existence of several stages in the development of the disease, separated by intervals of *latent* infection.) Every infectious process is the result of a guest-host relationship, and both the etiologic agent and the "terrain" have their importance. During the past half-a-century, the microbe (the "giving end" or extrinsic factor), i.e., the guest, had been overstressed. The time has come to place proper (not undue, or exclusive) emphasis on the terrain (the "receiving end" or intrinsic factor), i.e., on the host.

Even if contagiousness, pathogenicity, and virulence are qualifications of

the "invadi[illegible] microorganism, their definition must specify the "invaded" host, becau[illegible]k of proper identification could invalidate the intended meaning. The fa[illegible]ou[illegible] train H37Rv is avirulent (nonpathogenic) for reptiles. In this sense, f[illegible] rattlesnake's point of view, tuberculosis, however infectious, is not a "[illegible]g" disease. In the case of human beings, the contagiousness of the d[illegible]aused by *M. tuberculosis* is not fixed, but varies (13) during the epide[illegible]gic cycle of tuberculosis, in concordance with the changing proportio[illegible]une individuals among the population considered.

Is There Immunity in Tuberculosis?

[illegible] have become clear since Koch's first note (14) on his eponymic [illegible]on (suprainfection) phenomenon that tissue responses to specific anti[illegible] constitute the major, if not the only, cause for the appearance of tuberculous lesions. In the same article (and in previous publications), partial desensibilization attempts were heralded as the premonitory experimental basis for a biologic conquest of the disease. Koch's extract received instant world-wide attention, and was subjected at once to extensive trials; for instance, in this country it was first used on December 3, 1890, by John P. C. Foster (1847–1910); the vials had been brought from Germany by John H. Linsley (1859–1901); both of these physicians were themselves tuberculous. The world-wide failure,[2] not only as a vaccinating, but also as a therapeutic agent of what was to become the diagnostically priceless tuberculin (its "flare-up" effect is now being used to make tuberculous meningitis more amenable to chemotherapeutic attack), could not eradicate the initially established belief that the presence of live, preferably dormant, antigen somewhere in the body is a prerequisite for a state of immunity. This assumption, which is not entirely devoid of validity (at least from an experimental viewpoint) has persisted as a theoretical justification for BCG vaccination, notwithstanding the factual observation that the number of reactors decreases with the fall of morbidity and mortality from tuberculosis (16).

If negative reactability were to be equated with high susceptibility, every "open case" living in a community with a low tuberculin index should be surrounded by a corona of fresh tuberculous outbursts. Small-scale epidemics of this type have been occasionally reported (17), but they are the exception and could be due to a mutational boost in the virulence of the strains involved. As a rule, in recent years, particularly in this country, a comparatively large

[2] In 1908, at the Sixth International Tuberculosis Conference, in Washington, D.C., it was solely through the courtesy of presiding Flick that the aged Robert Koch (1843–1910) was allowed to exceed the time prescribed for discussions from the audience. At that meeting, Koch also made the statement that, contrary to his own former views (based mainly on the results obtained in the control of lepers in Norway), he had come to believe that isolation in tuberculosis is of little value, because in most instances the patient infects his surroundings before he is discovered (15). The Roumanian delegate to that conference, Stefan Irimescu (1874–1956), from whom the following story was learned in a personal communication, participated with Koch at a private banquet, offered by an industrialist from Baltimore; but neither the valuable gifts nor the exquisite meals presented to each of the twelve guests (selected among the delegates to the conference) pleased the somber, brooding, and visibly disillusioned Koch.

number of contacts must be examined before the survey will "yield" another case.

Immunity, meaning active resistance to microbial invasion, reproduction, and toxins, has been sometimes expressed as the titer of specific antibodies in the blood stream. Such a direct relationship may exist in certain infectious diseases (typhoid fever, cholera), but not in tuberculosis. It seems that for acid-fast organisms natural immunity is mostly a passive process, similar to the absence of reaction encountered after the inoculation of paratubercle bacilli, even when massive doses have been employed. This re-emphasizes the fact that virulence (damaging potential) of a given antigen is determined by the host's response: chromogenic microorganisms may at times kill a laboratory animal, and strains of Diplococcus isolated from the mouths of healthy human beings can be deadly for mice.

In the case of tuberculosis, susceptibility[3] may be construed as a low sensitization threshold on contact with tubercle bacilli (the reactability potentials of skin and of other organs are not necessarily synchronous nor of the same magnitude): the ulterior tissue-destroying process which results from the strong reactions to endotoxins (liberated after the death of a certain number of bacilli, due to limited local food supply and/or to the host's antibodies) will provide sufficient decay products to assure an adequate culture medium for bacterial survival, viz., for continuation of the vicious cycle. *Immunity against tuberculosis is the absence of reactability after repeated stimulation by specific antigens.* The degree of this immunity, whether innate or acquired, is inversely proportional to the potential magnitude of local and/or systemic tissue response. The existence of absolute immunity can be postulated to explain why some of the starved and emaciated World War II prisoners failed to develop clinical tuberculosis, despite prolonged exposure to contagious cases.[4]

Children reared by bacilliferous parents may never acquire a positive skin test (19, 20). In rare instances, even peroral (21) or parenteral (22) BCG vaccination, followed by intimate tuberculous contacts, will fail to produce the expected skin reaction. Two children, both of whose parents were tuberculous, received subcutaneous injections of "upwards of 600 virulent human tubercle bacilli" (23), but three years later they were negative to 100 per cent OT. In 1930, at Lübeck, 251 newborns received inadvertently enormous doses of live and virulent tubercle bacilli, instead of the intended BCG; 22 of the 251 never became tuberculin positive; if the 13 (of these 22), who were among the 77 (30.7 per cent) who ultimately died, are subtracted, there remain nine persistently tuberculin-negative, 6 of whom were also otherwise absolutely asymptomatic (24). Evidently, the concept of universal susceptibility to *M. tuberculosis* is out of order. On the other hand, a negative skin test, per se, does not imply

[3] The term susceptibility is used in preference to the anachronistic *predisposition*, which has survived by popular usage, even though modern biology should frown upon it, if only for its finalistic connotation.

[4] George E. Bushnell (1853–1924), who was both tuberculous himself and "the" tuberculosis specialist of the U. S. Army, often stated that "many of the tuberculized have an iron immunity, which no fatigue, hardship, or intercurrent disease can shake" (18).

immunity, because the respective individual (*a*) may not have been exposed to significant amounts of specific antigen; (*b*) his reaction could have changed; or (*c*) there might be a discrepancy between the antigenic response of his skin and of other tissues in the same body.

The transition between extreme susceptibility and absolute immunity is gradual, and in practice any intermediate step can be encountered. Moreover, the degree of local and/or systemic reactability (in a way analogous with the changing *peculiar malignant quality of the juices*, assumed by Marten in 1720,[5] may be altered in time. This would account for the protean character of the disease, a comparison first used in 1780 for scrofula (26). A good test case is the fact that corticotropin and cortisone, noted for their profound influence on tissue responses, may have a disastrous effect on the speed and extension of evolutive tuberculosis. It has been shown experimentally that stress can modify the secretion of corticosteroid hormones (27). This opens the way for speculation regarding the mechanism of psychogenic (war, emotional crises, depressive states) and physical (overwork, exhaustion) factors in phthisiogenesis. Conversely, when used along with antimicrobial drugs, corticotropin and cortisone have some merit in "cleaning up" exudative foci (28).

Is There an Hereditary Component in Phthisiogenesis?

With respect to the question of an hereditary component in phthisiogenesis several definite statements are carried in the Hippocratic Canon, for instance, the classical ἐχ φθινώδεος φθινώδης (29). The strength of the Coan mandate during its first two thousand years resulted in respectful acknowledgment of the inherited character of consumption in the teachings of every leading magister. Even those who advocated a contagious etiology[6] accepted heredity among the possible causes.

[5] Benjamin Marten is one of the least known forerunners of modern bacteriology. He believed that "the original and essential cause (of phthisis) . . . may possibly be some certain species of animalcula, or wonderfully minute living creatures" (25). It seems, however, that his approach in recruiting patients was not entirely orthodox, and he must have been subjected to all sorts of personal difficulties, because his preface contains a strongly worded paragraph, filled with indignant invective: "There scarcely was ever any book wrote and published, how good and correct in its kind so-ever, but what met with Opposition, Censure, or Ridicule from People of ill Nature, and of a cavilling Disposition, and that more especially if any Thing New or Uncommon was advanc'd in it. That the following Sheets therefore, should escape the splenetick Reflections of insidious Persons, is as far from my Expectation, as their Treatment of them and me, will be from sharing any part of my Concern."

[6] There is no mention of contagiousness in any of the works ascribed to Hippocrates (30), though phthisis is often discussed in *Epidemics*, especially in the first book, with emphasis on the so-called *epidemic constitution*, related to weather changes, either independently of, or connected to, a definite geographic site. A contemporary of Hippocrates, Isocrates (436–338 B.C.) composed the speech of a litigant heir, and used the risk involved as an argument in court to justify the will, by which the consumptive Trasylochus had bequeathed his fortune to the young man who had given him nursing care to the very end: this would certainly imply that contagiousness of phthisis was an accepted part of common knowledge. Since Aristotle (384–322 B.C.), who believed in contagion, was an ardent admirer of Hippocrates (460–370 B.C.), the pestilential doctrine was said to be, in spite of everything, of Coan extraction.

The term *familiae infectio* (31), used with reference to phthisis for the first time by Girolamo Cardano (1501–1576), must have originated from the unquestionable fact that tuberculosis very often shows a familial incidence. This obvious occurrence demanded an explanation, and resulted later in the concept of hereditary transmission of the contagious agent. Even the possibility of a facultative latency period and the skipping of one or more generations were admitted. The theme has a modern variation in the transplacental migration of the highly hypothetical submicroscopic (viral) stage of *M. tuberculosis*. The bacteriologic era proved that prenatal tuberculous infection can and does occur, but it has always been statistically insignificant. Its counterpart, the inherited susceptibility to contagion (the English lords give to their heirs the right to the peerage, not the peerage itself), called "condition precedent" by American authors (32), is all but a recent discovery: it can be found in the 1790 thesis of Francis-Bowes Sayre (33) of Trenton, and one century prior to that in the writings (34) of Franz de le Boë Sylvius (1614–1672).

The average medical reader of today regards old quotations as mere curiosities, preferably relegated to strictly historical papers. Quite to the contrary, antique sources are very helpful in placing the problem, and they often remind us that incorrect priority credits are imparted as frequently in the phthisiologic literature as in the remainder of the medical prose.

In practice, familial susceptibility to tuberculosis leads either to the extinction of the afflicted family, or else there will appear more or less immune decendants. This simple fact is very hard to translate in terms of contemporary genetics.

The predicament of the geneticist is similar to that of a mystic zealot, who must explain to skeptic bystanders why miracles, which in his doctrine are said to have occurred in the distant past, can never be identified within the palpable realm of documented history. After acknowledging that he has an ancestor in common with many living apes, the faithful devotee takes recourse to metaphysical elucubrations when he must explain why similar evolutions, even of infinitely lesser magnitude, have never been witnessed by recognized observers. Moreover, the geneticist incriminates the vernacular for perpetuating a confusion between biologic heredity and the inheritance of property, especially with regard to the phenotype. There is no "gene for blue eyes," i.e., regretfully no chance to find a gene for antigenic reactability; there is only a "marker" gene, which must "run" its full course within the framework of the entire genotype, and even then the intervening ecologic factors may contribute heavily to the final outcome. In this eclectic concept of "nature-nurture," the ecology serves as a safety outlet, since it can always be accused of having prevented the gene from reaching the intended mark. With regard to the basic problem of how the ecology can permanently influence the phenotype, the geneticists claim that, when genotype is altered by the environment, "the genes either continue to produce faithful copies of themselves, or they do not reproduce at all" (35).

Could all this be necessary only because we dare not face calmly our inability to authenticate experimentally the inheritance of newly acquired characters? How about BCG? They could not possibly demand that we provide an heretically finalistic tie-up between ecologic cause, genotypic modification, and phenotypic effect; or could they?

It might not be feasible to find a logical explanation for tuberculous facts within this Gordian maze of strings attached to the intricate constellations of markers and ecologic influences: with Alexander, we shall have to revert to fundamentals. It is difficult to comprehend why nature should have dispensed

with a mechanism by which immunity, when acquired during an infectious episode, could be transmitted to the progeny but, since it is controversial, such a possibility shall be deleted from our explanation. It is beyond reasonable doubt that susceptibility to tuberculosis runs in certain families and that the number of these susceptible families is visibly decreasing. It is also reasonable to assume that—similarly to what happens when insects are exposed to DDT or bacteria to antimicrobial drugs—resistant strains will develop as a result of the weeding-out of susceptible persons. Since modern genetic philosophy cannot furnish a satisfactory explanation for the facts about tuberculosis, in this paper, the hereditary component in phthisiogenesis shall be regarded as fully dependent upon straightforward, simple natural selection.

II. Theoretical Considerations Regarding the Epidemiology of Tuberculosis

It is a fitting tribute to the memory of a forgotten pioneer to place at the head of this section three quotations from one of the articles (36) of Harold Eugene Robertson (1878–1946), the former pathologist of the Mayo Clinic:

"The first that becomes pertinent is the ever-changing relationship between all hosts and all disease-producing parasites."

"While the wave character of acute contagious diseases seems to be a firmly grounded principle, the same tendency with respect to more chronic or widespread affections is either not recognized at all, or the application of this law is wholly denied."

"Disease cycles may be very long, they may even, although probably rarely, take centuries to finish their course, but in the end the wave *must* finish its rise, and inevitably fall, and the particular parasite as a group infection must, temporarily at least, cease to be a menace to its particular group host."[7]

The Secular Tuberculosis Curve

During epidemic episodes of short-term infections, such as measles or mumps, the morbidity and mortality rates follow a curve composed of two unequal segments (a shorter ascent and a longer descent), separated by a peak (acme). This aspect, known in mechanics as the *aperiodically damped motion*, is a basic trend of life (3). Adolf Gottstein admitted the wave character of tuberculosis rates (37) but, to prove it, the figures had to span a century or more, hence the qualification of *secular curve* (38). The unreliability of older (actually of any) statistics can be disregarded for this particular purpose. As long as one considers only the general tendency of the curve, it is of limited importance whether in a given city the peak of mortality was reached in 1780, 1800, or 1820, at a death rate of 800, 1,000, or 1,200.

[7] Robertson believed that the virulence of *M. tuberculosis* as a group parasite could in time decrease, which is contrary to logic. Even with such minor discrepancies, his dynamic approach to biology remains undeniable. A similarly realistic attitude was expressed by Allen E. Krause in 1928: "But a rising tide of tuberculosis cannot continue to rise indefinitely. As with every other infectious disease, there will be a limit somewhere."

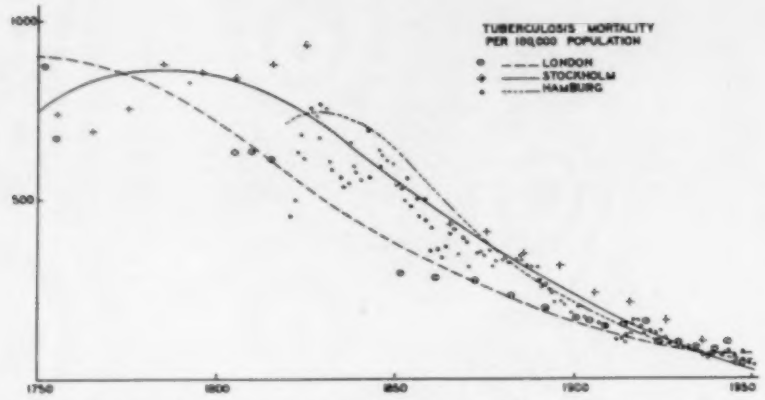

FIG. 1. Biologic phenomena exhibit cyclic patterns, and tuberculosis, as a group disease, makes no exception. The latest tuberculosis wave began its course in England in the sixteenth century, and reached a peak of mortality in London, probably before 1750. The capitals of Western Europe surmounted their peaks in the first half of the nineteenth century, those in Eastern Europe a few decades later. Statistical accuracy is wanting in early figures, but they permit identification of the general trend, which in this graph has been indicated by curves traced after considering the disparate points available. *Source:* municipal mortality records (*see* footnote 8); some data are taken from Clark (66) and Flatzeck-Hofbauer (38).

Long series for three European cities[8] have been included in figure 1, and ideal curves were traced to show the trend. This graph is a summary of the most significant segment of the Western European episode of the big tuberculous wave which swept the globe in the last centuries. Tuberculosis has been with us for a very long time, and its traces have been unearthed on repeated occasions (39). Previous waves might have been completed on a local scale, as expected with primitive transportation facilities. Earlier figures are not available, but it has been assumed that phthisis declined substantially after the barbaric invasions of Europe, and returned as a major disease (the latest wave!) following the re-population and socioeconomic regeneration of Christendom (40).

The existence of the tuberculosis wave can be fully explained only by the natural selection of individuals which were (and/or had become) insusceptible

[8] The data have been obtained through the courtesy of Mr. Olof Olinder, Assistant Director of the Stads Statistika Kontor in Stockholm, and of Mr. Lukoschus, Reg. Insp. of the Statistiches Landesamt of the Freien Hansestadt Hamburg. Dr. J. A. Scott, Medical Officer of Health of the London City Council, offered decennial figures beginning with 1851. In England, parish registers have been compulsory since 1538, yet the first longer series of vital statistical data appeared only in 1665 as *London's Remembrancer* by John Bell. His data, together with those of other pioneers, such as Graunt, Petty, Corbyn, Morris, and James Postlethwayt, were reprinted in 1759 in an anonymous tract, which contains crude mortality figures classified by causes of death, going as far back as 1629; but the difficulty is to locate sufficiently accurate population data for the computation of rates. This is why figures prior to 1750 have been omitted; but from the calculations it appears that the acme was reached around 1740 with about 900 deaths from tuberculosis per 100,000 population.

to the disease. This is corroborated by the study of *cohort mortality*, started by Andvoord (41), followed by Frost (42), and recently revived by Spicer (43). Werner (44) described a health resort which had received more than 170,000 tuberculous visitors, and these visitors had lived in rooms rented from the local population (at a time when the disease was believed to be inherited, and not contagious); yet the added number of bacilliferous individuals never interfered with the persistent and substantial downward trend of the tuberculosis death rate among the native inhabitants.

Virulence

Genuinely stable entities are unknown in the biosphere; therefore one must expect changes in the pathogenicity of *M. tuberculosis*. In fact, all acid-fast groups have probably a common ancestral strain.[9] A decrease in virulence is without consequence, as it appears inconceivable that this might occur simultaneously in the astronomic number of microorganisms required to influence the epidemiologic outcome. As a rule, increases in virulence are just as insignificant for the general phenomenon, although they can certainly shorten the life span of one or more persons, or even cause small epidemics (17). Under exceptionally favorable circumstances (46), a spontaneous, perhaps mutational, boost in acid-fast pathogenicity (added capacity to induce reactability) might crystallize into a "new" strain and originate another "wave."

Theoretical Development of a Tuberculosis Wave

Because mortality figures are more easily obtained and are to a certain extent universally comparable, death rates are still the common yardstick for evaluation of the tuberculosis phenomenon. Obviously, adequate appraisal requires many more data, but even experiments such as the famous Framingham (47) are too limited in time and space. Hence, calculated curves[10] will be used to explicate what happens when a "new" strain of boosted virulence invades a closed community. The specific mortality, i.e., the number of deaths due to tuberculosis divided by the total population, is actually the end result of several intermediate steps, such as the proportion of those who came in contact with (harbored for any length of time) the microorganism, of those among the contacts who became sensitized (infected), of those among the sensitized who developed the illness, and finally of those among the sick who succumbed to the disease.

[9] It is easy to agree with the statement of Maher (45) that "smegma bacilli to be found on the bodies of most people alive to-day are (not) the direct descendants of the smegma bacilli that were familiars of Cleopatra, and Charlemagne, and Alfred the Great, and Brian Boru." The selection of bearded chieftains is adequate, but in the case of Cleopatra, did he mean Caesar and/or Anthony?

[10] At this point it appears proper to quote the immortal Anatole France (in a free translation): "As a proof, fabricated evidence serves much better than authentic material, because it was prepared with a definite purpose in mind, and is therefore tailored to fit exacting requirements, with precision and specificity. It is also preferable because it offers an idealized concept, and thus diverts the mind from the mundane reality which, alas, is never pure."

Three of these intermediate proportions were selected for the tracings in figure 2. The tallest curve is the rate of "contacts" among the total population, a group easily defined by the term itself, and obviously fascinating for the theorist, but impossible to determine in practice. The middle curve is that of tuberculosis morbidity, a group which includes all of the tuberculin reactors, so as to avoid difficulties in definition. The bottom curve (inscribed on a different scale) is the tuberculosis death rate. Several intermediate curves might have been added; for instance, persons with clinical disease, "open" cases, "good chronics," inactive cases, et cetera, but their inclusion would only crowd the drawing; they all follow similar (interposed) tracings.

The exponential wave form is consistent with what can be expected when assuming a continuous natural selection of those immune to the disease. The steepness of slope of these curves is determined by the speed of spread of the "new" strain, which is in function of the number of contacts per unit of time.

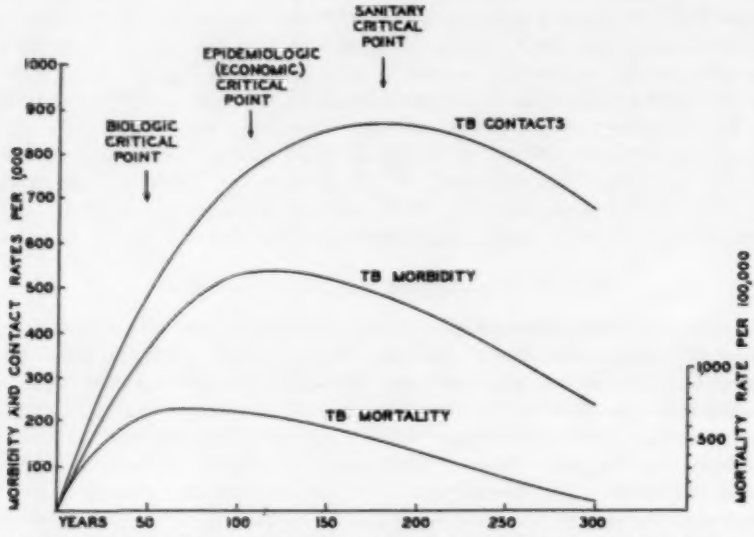

FIG. 2. This is the *theoretical* development of a tuberculosis wave in an imaginary community (with stable degree of urbanization, and population completely isolated from the rest of the world) in which tuberculosis is assumed to appear for the first time at moment zero. Shown are the death rate (inscribed on a different scale, at right), the rate of sickness, i.e., tuberculin-positive persons, which avoids complicated definitions, and the rate of contacts, all three by reference to living population. Other intermediate curves (persons with apparent clinical disease, "good chronics," convalescents, et cetera) were not included, as they would only complicate the drawing (all follow similar, interposed pathways) without adding new information. As expected with biologic phenomena (3), all of these curves consist of two unequal segments, the ascending limb of which is comparatively steeper and shorter than the exponentially decelerated descending leg. It is also characteristic that the peaks appear in succession, not synchronously, which in real life is apt to cause confusion and/or contradictory statements. Furthermore, since theoretically the curves cannot reach the base line before infinity, the incidence of tuberculosis may decrease to the level of medical curiosities, but it is not very likely ever to become "eradicated." *Source:* calculated data (*see* footnote 13).

The first curve which reaches its peak is the death rate, thus removing the threat of potential extinction;[11] this is the essential climax of the phenomenon, and may be called the *biologic critical point*. At such an early stage in the course of the wave, there are comparatively few immune persons among the population, and therefore the reactability index is still rising and will reach its summit at the *epidemiologic critical point*, which marks the transition from epidemic to endemic disease. As the wave advances, the "open" cases and the "good chronics" are proportionately on the increase and, even after they start their downhill course, the contacts are still climbing; but there comes a moment when the number of contagious patients is reduced to the point where even the contacts begin to drop off. This is the *sanitary critical point*, when the immunity of the population is so high that the disease ceases to constitute a public health problem. It is in fact the concluding climax, even though, just as in leprosy, the curves may never reach the zero line, e.g., there might always be a certain, however small, incidence of tuberculosis.

"Urban" and "Rural" Wave

The flatness of these curves, and the time span needed to attain a low level of tuberculosis incidence and mortality, are inversely proportional to the *degree of urbanization*. This cumulative term is here intended to include all those factors which increase either or both the opportunity of infection and the environmental stress, such as population movements, crowds, economic hardship (crammed living quarters, insufficient food intake), intercurrent epidemics, et cetera. War, with the added psychologic stress, is perhaps one of the most representative examples of acute urbanization. Extreme instances of "urban" and "rural" types of tuberculosis waves are shown in figure 3.

It was stated in the 1700s by the famous Suessmilch, and by his commentator Baumann (48), that phthisis seems to be more frequent among city dwellers than among farmers. At the beginning of the twentieth century, emphasis was placed on the different levels of rates found in industrial and in agricultural countries, to the point where it became almost an axiom that there must be

[11] There is no record of tuberculosis ever having really threatened the survival of any population. Peak rates beyond 1,500 per 100,000 are seldom encountered, and even those are below one-half or even one-third of the birth rate of that particular population. In fact, high tuberculosis death rates most often coincide with a positive demographic balance (birth rate exceeding the general death rate), while some of the lowest tuberculosis figures are found in aging nations, which have a negative balance (general death rate exceeding the birth rate). Extinction becomes a real threat only when the birth rate declines excessively. It seems that the birth rate is in proportion to, and obviously stimulated by the increase of, the death rate of the reproductive segment of the population. Wars provide in this respect unequivocal proof: the nations which had the largest losses in lives during World War II are today the strongest demographically, when compared with the other combatants. In fact, the people with the highest birth rate always win the *last* battle. These statements must not be construed as advocating war. Quite the opposite, they show the best way to reduce the birth rate, if this appears really desirable, by decreasing the death rate of the younger segments of the population considered. This viewpoint, however startling at first glance, is based on statistical evidence and other "cold" facts (3).

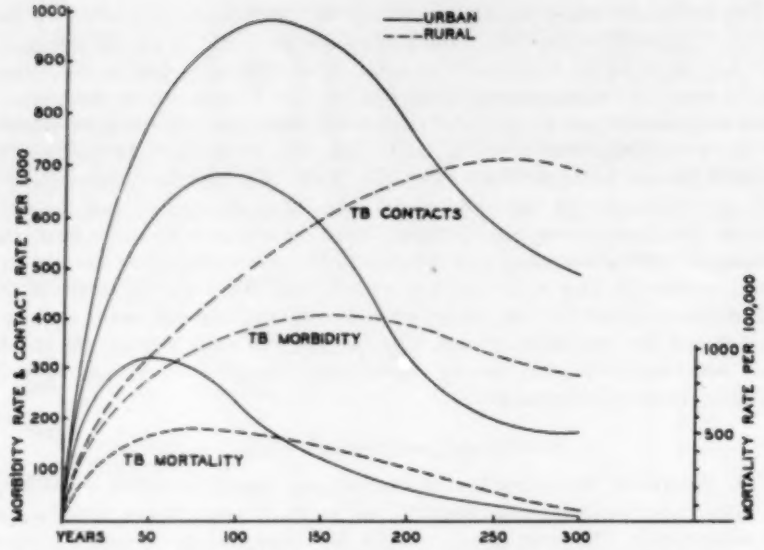

FIG. 3. Since the tuberculosis wave is the result of natural selection, its speed is proportional to the number of deaths per unit of time, and therefore, at any moment, the decrease will be commensurate with the preceding death rate. The pace of the wave is hastened by *urbanization* (*see* definition of this term in the text), because it increases the number of new contacts and raises the reactability potentials of exposed and afflicted persons (through stress and consequent alteration of corticosteroid secretions). Above curves represent the tuberculosis wave in two extreme settings (one urban, the other rural), these two imaginary communities being assumed to remain isolated from the rest of the world and to maintain a constant level of urbanization throughout the three centuries shown. These tracings, just like those in figure 2, are intended only as a general outline of the phenomenon and are not for numerical comparison with observed situations. The variation in death rates between countries, or between communities, can be fully explained by the differences in urbanization, both in time and space. An acute increase in urbanization, for instance, war or massive immigration from a less tuberculized territory, will result in a reversal of the downward trend, the added deflection acting as a superimposed miniature wave, with successive (not synchronous) miniature peaks of mortality, morbidity, contacts, et cetera. In point of fact, observed tracings, especially in the United States, contain as a rule multiple peaks of mortality. *Source:* calculated data (*see* footnote 13).

more tuberculosis in urbanized and industrialized, i.e., more civilized, than in rural and agricultural, i.e., more primitive, populations (49). Time has proved that this relationship was a transient situation related to certain phases of the tuberculosis cycle. Today many highly civilized, urbanized, and industrialized countries have a tuberculosis mortality below 10 per 100,000 population, while backward, less industrialized, eminently agrarian nations are still above 50. Likewise, certain metropolitan areas have reached lower levels than the surrounding rural or suburban communities; in the not too distant past, such occurrences have at times provoked anxiety, and public health officials were inclined

to offer even implausible explanations (50) in the hope of reconciling the facts with the prevailing doctrine.

Real figures can never produce the smooth curves depicted in figures 2 and 3 because in these imaginary, isolated communities, the population exhibits a stationary degree of urbanization. In real life, constant migratory movements are perhaps the most significant cause for fluctuations of all statistical rates in peace time. As a general rule, every newborn may be regarded as having received a level of immunity to (inability to react to the antigens of) tuberculosis, corresponding to the position on the tuberculosis cycle of his birthplace at the time of his birth. During life, this original level of potential reactability can be modified by intercurrent events but, generally speaking, the individual will maintain this degree of immunity throughout life, provision being made for changes occurring at different ages in the two sexes and other physiologic circumstances. These theoretical considerations can be extended to the entire population in a given community, which may then be regarded as having a mean level of immunity, corresponding to the statistical average of potential reactability of the individuals in the community. This concept has been confirmed by studies of cohort mortality (41–43).

The actual number of those who will develop reactability is dependent upon the level of immunity and the frequency and magnitude of exposure to specific antigenic stimulation. The percentages of those among the sensitized who will develop overt illness, and of those among the ill who are going to die from tuberculosis, are determined also by other factors (age, sex, localization, treatment, intercurrent diseases, et cetera), but the basic biologic, i.e., the most important and usually the deciding factor, is the underlying, presumably constitutional susceptibility or insusceptibility to potential reaction when contacted by the specific antigens. Obviously, between the two extremes there is a wide range of possible intermediate positions.

A higher degree of urbanization exposes a proportionally larger segment of the population to specific allergens, and thus comparatively more people participate from the beginning in the tuberculosis phenomenon (urbanization in itself causes added stress, which may alter the secretion of steroid hormones and thus influence the sensitization potential, and with it the clinical outcome in a statistically significant number of cases). The curves rise earlier, the peaks are higher and sooner reached, but also the selection is faster; consequently, the specific rates arrive at a lower level in a relatively shorter time span. Any increase in the degree of urbanization, whether sudden (war) or gradual (internal migration), speeds up the process and causes a transient hump on the curve, followed by an accordingly faster drop, due to the premature death of persons "scheduled" to get their share of tuberculosis and to succumb from it at a later date. A decrease in the degree of urbanization, for instance, substantial emigration, even from the cities to suburban or rural areas, causes initially a drop in the tuberculosis death rate; but afterwards the curve becomes flatter and will fail to decline as speedily as expected from its previous course.

The peak of mortality from tuberculosis occurred in London before the middle of the eighteenth century; in Stockholm, fifty to sixty years later. Most Western European capitals went through their tuberculosis climax in the first half of the nineteenth century, the respective country trailing its metropolis by several decades. The flat (rural) shapes of the Eastern European peaks started to materialize shortly after the middle of the nineteenth century. Before the end of the nineteenth century, most of North and South America had climbed over the

top of the tuberculosis hill and, at the beginning of the twentieth century, only a limited number of populations, located mostly in Asia and Africa, had still to reach their peak. In all civilized nations, at least in those which publish mortality statistics (if not all over the globe), tuberculosis death rates are today, for all practical purposes, on a downhill course. As there is a lag between the mortality curve and the intermediate curves (morbidity, sensitization, et cetera), for some time to come there will be areas with rising rates of morbidity and/or tuberculin sensitivity. Moreover, because of fluctuations, even transient increases in mortality rates can be encountered in communities in which the tuberculosis phenomenon is otherwise on the decrease. After all, a war will cause at least some spiking at almost any stage of the wave.

The Advancing Wave

It is doubtful whether any single individual can be immune against all infectious diseases but, since the human race is still flourishing on this globe, it is certain that so far no infectious agent has appeared on earth without there being individuals who either were naturally immune to it or developed immunity upon contact with that infectious agent.[12] In the case of tuberculosis, the percentage of immune individuals increases continuously from the very beginning of the wave. Every specific death eliminates a "reactable" individual, leaving behind a higher proportion of "less reactable" people. At the start of the wave, the toll of children and young adults is exorbitant; yet this also speeds up selection by removing a larger number of potentially reactable offspring. The average immunity of the total population must reach a substantial level to win the biologic battle, i.e., to *create* a peak of mortality. The rate of climb of the population's immunity is determined by the obstacle at hand: the mechanism is self-regulated because selection operates faster in the presence of a higher death rate (urban area), or slower, if there are fewer fatalities per unit of time (rural area).

The shapely curves in figures 2 and 3 are strictly fictional, and the rates[13] on which they are based do not represent averages for the critical points listed. Nor could standards ever be sensibly formulated because every peak is the result of local conditions, which

[12] Rabies *may* represent an exception, but adequate documentation is lacking, especially with respect to "natural" immunity.

[13] The curves in figures 2 and 3 have been calculated by using the formula $x = \alpha t e^{-\beta t}$ (3), in which x is the rate, t the time in years elapsed from the start of the curve, and e the base of natural logarithms (2.7183 . . .). In figure 2, the coefficient α is 38.04 for the deceased, 14.11 for the sensitized, and 13.96 for the contacts; while the coefficient β is 0.02 for the deceased, 0.01 for the sensitized, and 0.006 for the contacts. In figure 3, the coefficient α is 20.08 for the rural deceased, 7.63 for the rural sensitized, 7.61 for the rural contacts, 65.33 for the urban deceased, 23.86 for the urban sensitized, and 23.60 for the urban contacts; while the coefficient β is 0.015 for the rural deceased, 0.007 for the rural sensitized, 0.004 for the rural contacts, 0.025 for the urban deceased, 0.013 for the urban sensitized, and 0.009 for the urban contacts. For instance, the peak of the urban death rate in figure 3 is reached at forty years after the beginning of the wave: the formula is $x = 65.33 \times 40 \times 2.7183^{-0.025 \times 40}$ = approximately 962; that is, 962 deaths per 100,000 population.

vary with time and place. As postulated, the imaginary curves occur in an isolated community, which had a stable degree of urbanization. When examining true tracings, it is not unusual to find that fluctuating migratory movements and uneven urbanization can and do produce more than one essential climax. The moment at which the most prominent peak is reached, and its height, will be determined by the timing of the urbanizing pulse in its relationship to the phase of the tuberculosis wave: the impact of urbanized tuberculosis is inversely proportional to the average level of immunity of the population afflicted. If London had to escalade its main spike in the twentieth century, the toll would be disastrous; the spread of the disease should mimic the thoroughness of influenza epidemics, but it could exhaust itself in a few decades instead of centuries, and the over-all number of casualties might be smaller. This surprising fact, easily verified by integral calculus in the "perfect" curves in figure 3, seldom obtains in nature because fresh contingents of sensitizable persons keep migrating to the cities. However, among stationary populations, the totals of afflicted (either sensitized and/or dead) persons must be larger, in the long run, under rural than under urban circumstances.

All of the aspects of the tuberculosis phenomenon change as the wave progresses. The rising level of immunity lengthens the average life span of the tuberculous patient. Therefore, five-, ten-, and twenty-year survival rates should be used with extreme caution in assessing the value of specific treatment methods. The observer, with patients coming from a more urbanized area, will have better results because his figures reflect a comparatively higher level of immunity. Even totals obtained from randomized studies performed at institutions scattered over a large territory with a heterogenous population are not entirely fool proof, since it is not certain that randomization must homogenize the heterogeneity. Perhaps the most fallacious are comparisons made between successive periods in which the same therapeutic procedures have been employed: these always demonstrate noticeable improvement of the results (51). This pitfall is not always recognized (52).

Considering only schematic essentials, in a given case of tuberculosis, either the localized (productive) or the generalized (hematogenous) character predominates. From this viewpoint, generalized forms are more often seen at the beginning of the epidemiologic wave, localized types toward its end. This progressive escape from tuberculosis of increasing segments of the population has been demonstrated in autopsy material, analyzed in 1945 (53), and compared with similar series, compiled in 1943 (54) and in 1917 (55). A slightly different approach was used by Medlar in 1948 (56), but they all refer to the figures established in 1900 by Naegeli (57). Actually, the first modern study of this type was published in 1893 by Schlenker (11), with the intent to promote the concept of the universalism of tuberculous infection. The princeps article of this type in America was published in 1842 by Jackson (58), who analyzed 604 autopsy cases collected during ten years in and around Boston; unfortunately the figures are not broken down by age groups, which makes comparison with later data very difficult, if not impossible.

Even the patterns of childhood tuberculosis are constantly changing (59). Today, cavities are not as infrequently seen in children (proportionally!) as they were at the turn of the century, although now tuberculosis is much rarer

in the pediatric group, both absolutely speaking and by comparison with other age groups. The apparent increase in the specific tuberculosis mortality of senior citizens (mostly males) results from their relatively lower average immunity level, i.e., the level of their cohort. The comparatively lower death rate of older females only confirms the truism that there is also a biologic, not only a physical, difference between the sexes (60).

Tuberculosis Mortality at Various Ages in the Two Sexes

The literature contains many peculiar interpretations of what were thought to be constant features among the numerous variations in the analysis of tuberculosis death rates by age and sex. A number of so-called characteristics proved to be only transient-phase aspects, related to the development of the tuberculosis wave; for instance, the well-publicized dip in the five- to fourteen- year age group, regarded as a transitional stage between the generalized forms of the newborn and infant and the localized or reinfection types of post-adolescence. As a matter of fact, a very shallow dip can still be identified in today's tracings, but only with the help of optic or graphic magnification. An apparently constant feature (occasionally obscured in recent years because of the over-all small, statistically insignificant absolute figures in the very young) is the higher tuberculosis mortality of the male at the two extremes, in the newborn and in the very old. Conversely, around twenty years of age, there is a definite prevalence of tuberculosis in the female. The latter finding seems to have been known to Hippocrates (61).

The stylized curves in figure 4 illustrate three extremes. The top tracings are encountered in the epidemic stages of the tuberculosis wave and indicate an over-all high rate of specific mortality, the only light spot being the five- to fourteen-year dip. The rates in the older age groups often resemble a pseudocarcinomatous curve (rise continuing with increasing age), more pronounced in the male.

While the above described aspect is consistent with a poor average immunity, the bottom tracings are those expected toward the end of a tuberculosis wave, showing a high immunity of the entire population, but especially of the younger generations. The older age groups, corresponding to earlier cohorts, have a lower immunity and will exhibit a pseudocarcinomatous trend, which is steeper if the wave has run a fast course (speedy urbanization); otherwise it should show a more gradual slope.

The middle tracings, interposed between the initial and final stages of the wave, correspond to an intermediate phase, in which the base population has reached a fairly adequate immunity (although not as far advanced as in the late stages of the wave), while the main "fodder" for tuberculosis mortality is provided by immigrants, either from rural areas and/or from less tuberculized foreign countries. These immigrants are generally young people, and they and those of their children who are still reactable furnish the quota needed to fill the supercharged (overlaid) mortality in the zero- to one- and the twenty- to thirty-four-year age groups, so characteristic of this stage of the tuberculosis wave.

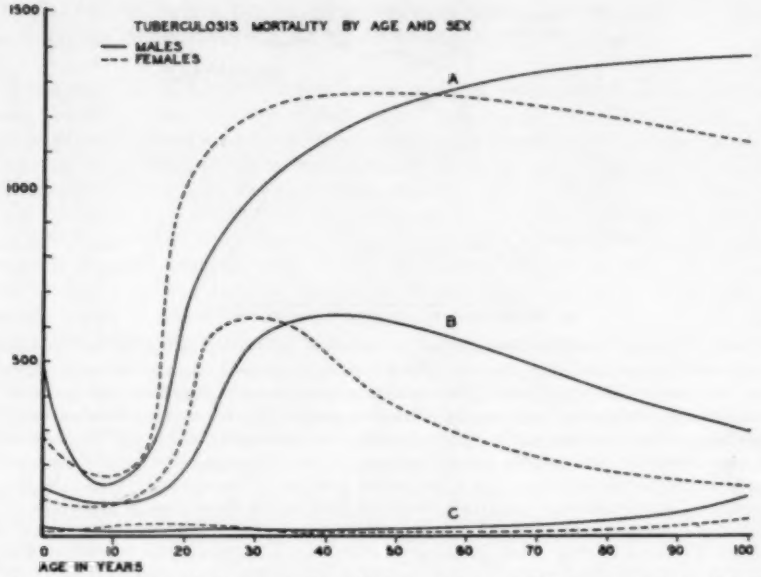

FIG. 4. In this *theoretical* presentation of tuberculosis mortality by age and sex, *A* is the situation at the height of the epidemic period, *B* at an intermediate stage, and *C* at the end of the wave. Tuberculosis mortality in the fifteen- to twenty-four-year-old female is always higher than in her male contemporary (thus far, there exists no valid explanation for this fact). Male mortality from tuberculosis seems to prevail in the newborn and in the very old. The five- to fourteen-year "dip," so outstanding at the start, barely survives as a shallow remnant. The pseudocarcinomatous appearance in the aged, at the end of the wave, is steeper when the wave has had a hasty course.

Within this framework there are many possible variations, such as delays or speed-up, overlapping stages, et cetera, but with some analysis most of them can be reduced to the three essential phase aspects above described.

Race and Tuberculosis

Considerable efforts, much ingenuity, and many pages have been devoted to the study of this problem in the attempt to prove that certain races are especially susceptible to *M. tuberculosis*. Some investigators have even earnestly searched for possible metabolic affinities between the degree of cutaneous pigmentation and the acid-fast cuticle of the microorganism. Racial susceptibility cannot be denied as a theoretic possibility, though it does not appear to be needed to explain observed facts. In this respect, it seems that "optimal tuberculization" (18) is the most important prerequisite for ultimate victory, knowing that the descendants will be the victorious ones, following a heavy death toll paid by the ancestors. These arguments shall be further substantiated in the next section. Under the circumstances, it is up to those who believe in a peculiar racial susceptibility to present convincing evidence for their suppositions. To this

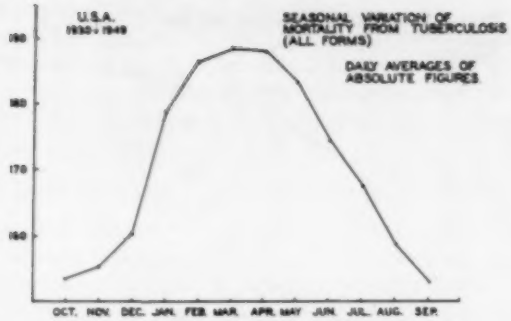

FIG. 5. In the Northern Hemisphere, tuberculosis mortality is highest at the vernal, and lowest at the autumnal, equinox. The situation is reversed south of the equator, where the seasons are also transposed. The variations do not exceed 20 per cent of the total (at the maximal deflection) and appear magnified graphically for better visualization. The constancy of this seasonal variation has never been satisfactorily explained. The asymmetry of this otherwise bell-shaped, almost frequency type of, curve (convexity of descending limb, as compared with concavity of ascending portion) is due to the fact that the top incidence of extrapulmonary localizations occurs from one to three months after that of the pulmonary form (*see* figure 6). The seasonal variation in the appearance of new cases and of reactivations is even more pronounced than that of tuberculosis mortality. *Source:* National Office of Vital Statistics (for method of calculation, *see* footnote 14).

date, the differences in tuberculosis incidence, in clinical forms, in skin and other reactivity, in death rates, et cetera, between two or more races, are all easily explained as phase aspects within the expected development of a tuberculosis cycle.

Meteorologic Influences in Tuberculosis

Weather factors have received little attention in the medical literature of recent vintage, mostly because very little is known about the physiopathology involved and because the opinions expressed contained audible empiric overtones. Among the lesser investigated aspects of tuberculosis is the seasonal influence on its development. It was known that phthisics succumbed more often in late winter and early spring than at any other time of the year (62, 63). A periodogram (figure 5),[14] based on absolute daily average mortality figures,

[14] The data were calculated as follows: In January, 1930, 7,410 persons died from tuberculosis in the United States; to these were added the 7,109 persons who died with this diagnosis in January, 1931, and so on until and including January, 1949; the total of 110,776 deaths which occurred in the twenty months of January, 1930–1949 were divided by 620 (twenty months times thirty-one days each) days, and the result, 178.7, represents the daily average of deaths due to tuberculosis in January, and was thus inscribed on the graph. A similar calculation for February, 1930–1949 gave 105,263 deaths, which were divided by 565 (twenty times twenty-eight days to which were added five bissextile days for the leap years) days resulted in 186.3 average daily tuberculosis deaths in February.

reveals that the highest rate of tuberculosis casualties coincides with the vernal equinox; the lowest, with the autumnal equinox.[15]

Acute tuberculosis episodes, such as new infiltrations, exacerbations, or reactivations, show a similarly higher incidence in early spring. This has been stressed before (63), but few people know about it, and even fewer give it enough consideration to time their surveys accordingly. In state mental institutions in Illinois, all patients and employees have a chest survey film about every six months. After six years of experience with these surveys, the actual dates of which are distributed more or less evenly throughout the year, this writer may attest to the fact that the yield of new and reactivated cases was significantly larger in spring and early summer than in any other season. The increased incidence in early summer could be explained by the fact that it takes a few weeks to a few months before the lesion becomes visible. Spontaneous off-season reabsorption of some of the infiltrates may also account for their relative accumulation during the favored season.

As of today, there exists no valid explanation for these facts and figures. One may take recourse to a term endeared to French and Italian authors, and speak of the relationship between tuberculous activity and neuro-vegetative lability. As it happens, the autonomic system is disturbed in practically every disease, after every meal, and even after every argument, so that use of this explanation amounts to hiding behind a fancy name. Tchijewsky, a physicist who dedicated a lifetime of research to elucidate the connections between meteorology and medicine, believed in the existence of a causal relationship between sun-spot periodicity and many historic events. While such a possibility cannot be denied, the evidence presented was, by its very nature, strictly circumstantial. He also contended that the important element is atmospheric electricity and, since this is as vague and evasive a term as health, he singled out atmospheric ionization as the real culprit. Subsequently, Tchijewsky (64) suspended ionizing wires[16] in the dormitories of some tuberculosis sanatoriums in Russia.

Atmospheric ionization is a very elusive phenomenon, subject to astounding changes. In a room, there may be significant differences between a window location, the bed, or the floor. Outside, there are even more important discrepancies between the measurements obtained on concrete pavement, earth, vegetation, metallic roof, et cetera. Daily variations, even independent of meteorologic phenomena, are also observed, and differences of several hundred or thousand times are not unusual. After considering all of these physical hindrances to the establishment of a base line for comparison, the statement is offered that ionization is supposed to influence the tuberculous process by way of the neuro-vegetative system. Such a relationship is theoretically possible, but so far this assumption has not progressed beyond the stage of philosophic speculation.

[15] In the Southern Hemisphere, where the seasons are transposed, tuberculosis mortality is also reversed; for instance, in Buenos Aires more tuberculous die in September, the lowest rate occurring in March. As it would have been of interest to determine what happens at the transition point (equator), repeated though unsuccessful attempts were made to obtain by correspondence monthly tuberculosis mortality figures for the state of Ecuador and/or for its capital Quito.

[16] The 1957 Philco air-conditioner has been advertised with an optional (promotional!) device, which could deliver not only cooled, but also ionized air. The *ionic surgery* advocated by George Betton Massey (1856–1927) for the treatment of tuberculous adenopathies (also for cancer and hemorrhoids) was Zn-Hg iontophoresis.

A personal observation, even more difficult to explain, is the fact that not all tuberculosis localizations have their maximal incidence at the same time. In fact, only the respiratory forms reveal a high mortality at the vernal equinox. Analysis by separation of respiratory and extrarespiratory tuberculosis mortality (figure 6)—the latter, which amounts to about 10 per cent of the former, had to be multiplied by ten for adequate comparison—reveals that there are at least two additional peaks, one in April or May, caused by meningeal and miliary tuberculosis; the other in June or July, due to urogenital forms. The reason for—or at least the mechanism of—this delay could provide a welcome insight into the pathogenesis of tuberculosis.

Emancipation of Clinical Tuberculosis

The influence of these seasonal factors decreases as the wave advances, and the vernal spikes become less pronounced. In the late stages of the cycle, tuberculosis as a clinical entity becomes increasingly independent of extraneous stimulation. World War II, which was harder on the Central European countries than World War I had been, resulted, nevertheless, in a substantially smaller "hump" on their mortality curves, because the second war occurred at a time when their average level of immunity was higher. It seems that even the rest cure gives better results at the height of the wave. There might be some connection between this observation and the reversals in prescribing rest or exercise for phthisics, witnessed in the course of medical history.

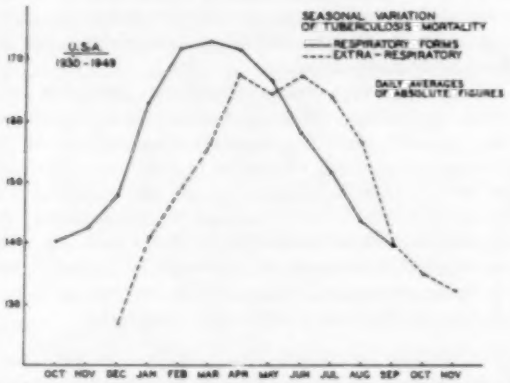

FIG. 6. Extrapulmonary localizations (which represent only about 10 per cent of the total tuberculosis mortality) have been inscribed as if multiplied by ten to allow for better interpretation. There are at least two spikes of extrapulmonary tuberculosis mortality, the first of which occurs about one month after the vernal equinox, and is probably due to meningitis and miliary forms. The second extrapulmonary spike takes place after another two months (approximately), and is believed to include the urogenital forms. If the actual reason for these delays could be found, it would certainly further our understanding of phthisiogenesis. *Source:* National Office of Vital Statistics (for method of calculation, *see* footnote 14).

In civilized nations, the rapid industrialization has resulted in a speedy course of the tuberculosis wave, which has made seven-league strides and is now many decades ahead of what it would have been without the wars and the repeated industrial revolutions. The cycle is so far advanced, and the tuberculosis has become so emancipated, that many patients reach a stage in which

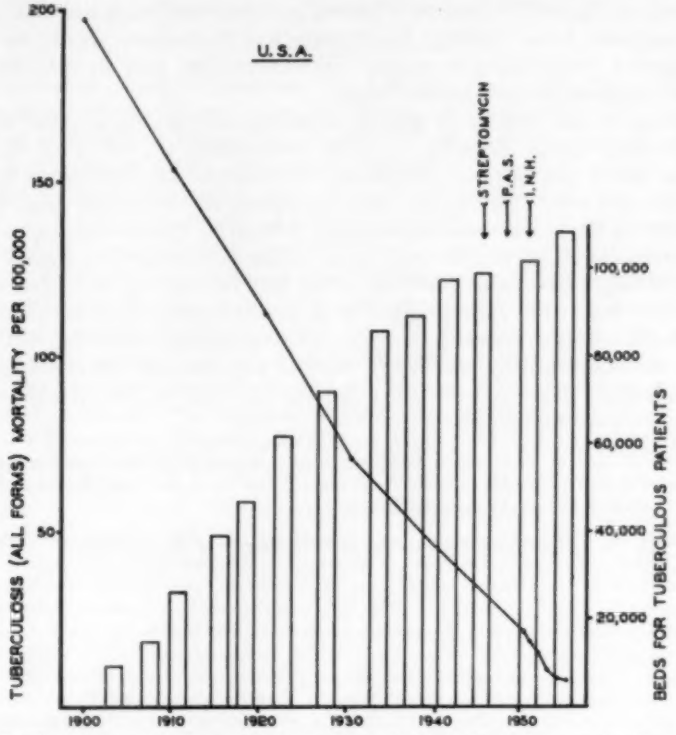

FIG. 7. The decrease in tuberculosis mortality coincides with an increase in the number of tuberculosis beds, but there seems to be no causal relationship between the two series. In fact, the decrease of the death rate shows a slight deceleration, which is *not* due to a contra-selective effect of the antituberculous program (the number of active cases, about 250,000 according to the 1957 estimate, is twice the total number of beds available for tuberculous), but to the fact that in its logarithmic course the death rate must level off, as it will reach the base line only at infinity. The advent of antimicrobials was likewise unable to modify the pathway of the death rate from tuberculosis, which is not unexpected, since the more effective penicillin and broad-spectrum antimicrobials have not yet modified the course of the death rate from pneumonia. Nevertheless, in the future, when the absolute figures of tuberculosis as a group disease will be lower, and the specific antimicrobials more effective, the time may come when the death rate could be influenced by these factors. *Source:* National Office of Vital Statistics and National Tuberculosis Association (*see also* footnote 17).

their disease is burned-out (cured ?), sometimes despite extensive mutilating procedures to which a fair number of them have been subjected in the last decades. The effectiveness of streptomycin, PAS, and isoniazid and of other compounds has been demonstrated beyond reasonable doubt, both in individual cases and in serial clinical trials. So far, however, none of these "wonder drugs" has influenced the mortality rate from tuberculosis, which is slowly leveling off (figure 7),[17] because it follows an exponential track and cannot reach its base level before infinity. Small statistical fluctuations should be wisely disregarded, yet piddling over minor differences from year to year (65) may have been going on since before Graunt.

Because of the leveling-off process, a malevolent soul might argue that the antimicrobial drugs, the clinics and the sanatoriums, as well as all the other public health procedures, have stalled the decrease of tuberculosis by their anti-selective effect. This is very hard to believe, in view of the deep, biologic causality of the tuberculosis phenomenon, difficult to influence during its phases of high incidence. As the absolute figures continue to decline, it is possible that at some future date an anti-selective effect may be observed. It is also conceivable that large-scale chemotherapy with more effective drugs could influence the death rate from tuberculosis. It must be emphasized, however, that to this date, the comparatively much more effective penicillin and the broad-spectrum antimicrobials have likewise failed to alter the slope of the (also decreasing) death rate from pneumonia.

[17] The number of tuberculosis beds and recent mortality figures have been obtained through the courtesy of Mr. Donald A. Trauger, Director of the Social Research Division of the National Tuberculosis Association.

(*To be continued in the next two issues of the* REVIEW)

A SIMPLIFIED METHOD FOR ESTIMATION OF THE DIFFUSING CAPACITY OF THE LUNG IN SUBJECTS WITHOUT AIRWAY OBSTRUCTION[1,2]

M. HENRY WILLIAMS, JR., AND LENORE ZOHMAN[3]

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INTRODUCTION

Measurement of the diffusing capacity of the lungs for carbon monoxide (D_{CO}) is becoming an increasingly important method for assessment of pulmonary function in clinical medicine. Calculation of the diffusing capacity by the steady-state method described by Filley and co-workers (1) requires knowledge of the alveolar carbon dioxide tension ($P_{A_{CO_2}}$) for calculation of the lung dead space and, hence, of the partial pressure of carbon monoxide in the alveolar air ($P_{A_{CO}}$). Since the $P_{A_{CO_2}}$ rarely departs markedly from a value of 40 mm. of mercury in subjects without airway obstruction unless lowered by hyperventilation, it is conceivable that the D_{CO} could be estimated with sufficient accuracy in such patients by assuming either a normal value for $P_{A_{CO_2}}$ or for the ratio of dead space to tidal volume (Vd/Vt). This report compares the value for D_{CO} calculated from the carbon monoxide uptake, making each of the above assumptions to the value obtained by the original method. Measurements were made during muscular exercise in order to approach a maximal value and in order to minimize the effects of an erroneous assumption of $P_{A_{CO_2}}$ or Vd/Vt.

MATERIAL AND METHODS

Twenty-one patients referred consecutively for evaluation of pulmonary function in whom airway obstruction was minimal or absent, as evidenced by a maximal midexpiratory flow rate of more than 1.0 liters per second (2), are included in this report. Studies were done during muscular exercise, which consisted of pedalling in the recumbent position against a fixed resistance at an estimated or predetermined maximal rate for five minutes. Throughout the period of exercise, the patient breathed a known concentration of approximately 0.1 per cent carbon monoxide in air. During the last two minutes of exercise, expired air and arterial blood samples were collected. Expired gas composition (Scholander) and volume were measured, and the carbon monoxide content was determined on a carbon monoxide gas analyzer.[4] Arterial blood was analyzed for pH (glass electrode) and carbon dioxide content (Van Slyke), and the arterial P_{CO_2} was calculated. Carbon monoxide uptake was calculated from the expired gas composition and volume, and three calculations of $P_{A_{CO}}$ were performed: (*1*) using the $P_{A_{CO_2}}$ as determined by arterial blood gas analysis, (*2*) assuming a normal $P_{A_{CO_2}}$ of 40 mm. of mercury, and (*3*) assuming a Vd/Vt ratio of 40 per cent, which was the average value found in these patients. The diffusing capacity was then calculated for each $P_{A_{CO}}$.

RESULTS

In table 1 are listed the diagnoses, ventilatory data, and measurements obtained during exercise of oxygen consumption, arterial blood gas composition,

[1] From the Cardiorespiratory Laboratory, Grasslands Hospital, Valhalla, New York.

[2] Supported in part by grants from the Westchester Heart Association and the Westchester Tuberculosis and Health Association.

[3] Fellow, Westchester Tuberculosis and Health Association.

[4] Liston Becker gas analyzer Model 15-A.

TABLE 1

DIAGNOSIS, VENTILATORY DATA, AND MEASUREMENTS OBTAINED DURING EXERCISE OF OXYGEN CONSUMPTION, ARTERIAL BLOOD GAS COMPOSITION, EXPIRED P_{CO_2}, AND DIFFUSING CAPACITY OF TWENTY-ONE SUBJECTS

Name	Diagnosis	Vital Capacity	Maximal Mid-expiratory Flow	Oxygen Consumption	Arterial Oxygen Saturation	Arterial P_{CO_2}	Expired P_{CO_2}	Diffusing Capacity cc/min/mm. Hg		
								True	Estimated for:	
									$P_{A_{CO_2}}$ = 40	Vd/Vt = 40%
		per cent	*l/sec.*	*cc/min.*	*per cent*	*mm. Hg*	*mm. Hg*			
Spe	Sarcoidosis	49	2.01	496	67	34	14	4.4	4.6	4.1
		64	3.26	432	84	40	19	5.9	5.9	4.3
		79	1.61	608	66	36	19	5.4	5.5	5.4
For	Sarcoidosis	44	7.01	561	90	39	22	9.1	10.1	9.8
		52	2.73	545	98	50	22	10.9	7.6	9.5
McK	Sarcoidosis	40	2.16	419	89	42	24	12.3	11.5	30.9
		40	1.45	518	93	43	28	10.0	7.1	23.1
		—	—	427	84	37	26	10.8	11.9	21.2
Gul	Fibrosis	103	6.90	537	93	37	16	8.8	9.2	7.8
Nea	Sarcoidosis	51	2.42	301	66	23	11	12.1	35.0	13.0
Bur	Tuberculosis	66	1.06	606	87	39	26	35.3	38.0	66
Ada	Asthma	80	1.32	763	95	43	25	16.4	15.2	18.9
Pau	Bullae	90	1.31	815	96	41	24	9.9	9.7	15.4
Leg	IASD*	—	—	350	90	28	14	10.7	13.0	10.7
Sch	ASHD*	90	1.60	704	94	45	24	18.1	16.3	17.9
Haw	ASHD	86	1.87	653	92	48	27	27.3	24.4	27.3
Sex	R.H.D.*	—	—	625	93	45	33	23.8	19.3	68
Gal	R.H.D.	59	1.00	471	88	44	22	15.7	13.8	16.1
Tay	R.H.D.	75	2.91	341	95	33	17	14.9	23.4	19.5
Min	R.H.D.	89	1.56	310	84	42	10	4.8	4.5	3.3
Jin	R.H.D.	43	1.36	450	95	36	18	7.3	7.7	7.3
Atk	R.H.D.	94	3.37	1,020	93	39	28	23.1	23.9	35.3
Bal	Nephritis	74	2.32	960	98	40	26	28.6	28.6	39.8
Pom	Asthma	90	1.25	615	95	42	24	17.0	16.4	17.6
McK	Asthma	87	3.94	915	90	46	27	19.4	17.6	21.2
Con	R.H.D.*	79	1.50	449	97	30	19	17.2	30.0	22.2

* IASD—Intra-atrial septal defect
ASHD—Arteriosclerotic heart disease
RHD—Rheumatic heart disease

expired carbon dioxide tension, and diffusing capacity. The maximal work which could be done by these patients varied. Patients *McC*, *Bal*, *Atk*, and *Pau* had exercise oxygen consumptions exceeding 800 cc. per minute, whereas patients *Nea*, *Leg*, *Tay*, and *Min* consumed less than 400 cc. per minute. The diffusing capacity ranged from 4.4 to 35.3 cc. per minute per mm. of mercury, thereby providing a wide range of values for comparison.

The relationship between diffusing capacity calculated from the directly measured carbon dioxide tension and the value obtained when $P_{A_{CO_2}}$ was assumed to be 40 mm. of mercury is demonstrated in figure 1. Although agreement was

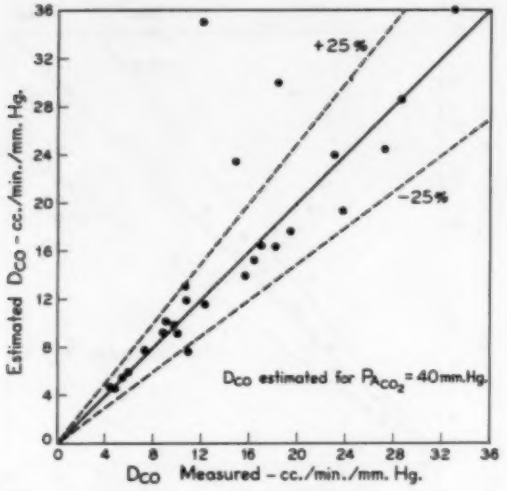

FIG. 1. The relationship between the diffusing capacity calculated using the measured arterial carbon dioxide tension (abscissa), and the value estimated when $P_{A_{CO_2}}$ was assumed to be 40 mm. of mercury (ordinate).

good in most instances, 3 patients revealed a very high estimated D_{CO}. This discrepancy resulted from the fact that the arterial carbon dioxide tension was lowered by hyperventilation so that the true $P_{A_{CO}}$ was higher than the estimated value. In figure 2, the D_{CO} calculated from the directly measured arterial P_{CO_2} is compared with the value obtained when Vd/Vt was assumed to be 40 per cent. Agreement between the estimated and true values was not as good as that shown in figure 1.

Of the two methods, estimation of the D_{CO} for a normal $P_{A_{CO_2}}$ appeared to be the more accurate method, particularly if patients with hyperventilation were excluded. In the presence of hyperventilation, which was accompanied by reduction of the expired carbon dioxide tension ($P_{E_{CO_2}}$), values obtained using the alternative method for the estimation of D_{CO} more closely approximated the true values. In figure 3, D_{CO} is plotted against the value estimated for a $P_{A_{CO_2}}$ of 40 mm. of mercury when the $P_{E_{CO_2}}$ was 20 mm. of mercury or greater, and is plotted against the value estimated for a Vd/Vt of 40 per cent when $P_{E_{CO_2}}$ was less than 20 mm. of mercury. It is apparent that, by selecting the method of calculation in this way, the diffusing capacity may be estimated in most instances within 25 per cent of the true value.

DISCUSSION

From the data presented, it appears that the method described above provides a close estimation of the diffusing capacity of the lung in subjects without significant airway obstruction. The method is not valid in the presence of greatly

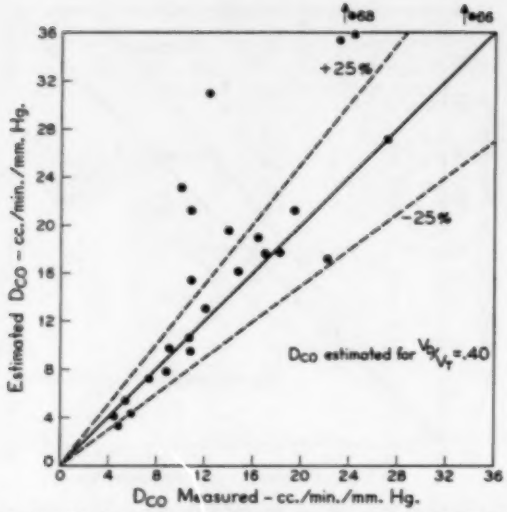

FIG. 2. The relationship between the diffusing capacity calculated using the measured $P_{A_{CO_2}}$ (abscissa) and the value obtained when Vd/Vt was assumed to be 40 per cent (ordinate).

elevated arterial carbon dioxide tension, such as may occur in the alveolar hypoventilation syndrome associated with obesity or disease of the chest wall (3). The most common cause of elevated carbon dioxide tension, airway obstruction, can be excluded by spirometry. Despite the limitation to use in patients with normal or increased alveolar ventilation, the reported method of estimating D_{CO} has practical application in several diagnostic and therapeutic situations, and obviates the necessity for arterial puncture.

Cugell and associates (4) have shown that patients with shortness of breath because of pulmonary fibrosis are characterized by a low diffusing capacity which is inversely related to the degree of disability. Such patients need not manifest abnormality of arterial blood gases or of ventilatory function, although hyperventilation is common. The patient *Gul* in the present series is an example of the way in which the availability of a rapid and simple method for determining the diffusing capacity of the lung may clarify a pulmonary diagnostic problem.

A 48-year-old mechanic had one-flight exertional dyspnea subsequent to moderately advanced pulmonary tuberculosis treated with pneumoperitoneum and antituberculous drugs three years previously. Minimal pulmonary fibrosis was shown roentgenographically, and arterial blood gases and ventilatory studies were normal. Normal pulmonary arterial pressure and cardiac output at rest and during exercise, determined by cardiac catheterization, excluded a cardiac basis for his dyspnea.

The patient's symptoms were thought to be functional until a very low diffusing capacity was measured during both rest and exercise. This was the only de-

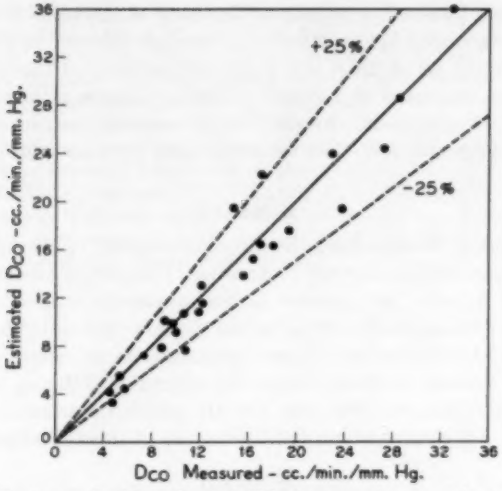

FIG. 3. "True" compared with estimated diffusing capacity assuming a $P_{A_{CO_2}}$ of 40 mm. of mercury when $P_{E_{CO_2}}$ was mm. of mercury or greater, and a value of 40 per cent for when $P_{E_{CO_2}}$ was less than 20 mm. of mercury.

tectable abnormality of pulmonary function and confirmed the diagnosis of pulmonary fibrosis, possibly secondary to tuberculosis. The absence of hypoxemia in this patient was not surprising, since very extensive impairment of diffusion must be present before the arterial oxygen saturation falls below 90 per cent (5). In addition, it is of interest that pulmonary fibrosis produced marked diffusion limitation in this patient, but no measurable encroachment on the pulmonary vascular bed.

Preoperative evaluation for resectional chest surgery may be assisted by the measurement of diffusing capacity. In the experimental animal, reduction of the diffusing capacity is a critical factor in the amount of lung tissue which may be removed without resultant respiratory insufficiency (6). Consequently, this measurement may prove to be of prognostic value in patients being considered for pulmonary resection.

It is possible, with this technique, to determine the D_{CO} in severely disabled patients, since, even when the level of oxygen consumption is low during mild exercise, there is good agreement between the estimated and true values. The close agreement of estimated and true values for D_{CO} which may be obtained in severely incapacitated patients is illustrated in patients *Nea* and *Spe*, both of whom had severe pulmonary hypertension and could perform only mild exercise.

Bates has recently re-emphasized the importance of measuring the diffusing capacity in order to distinguish between bronchial asthma and pulmonary emphysema (7). In the absence of severe airway obstruction, the above-described technique may provide this distinction. In addition, this technique is

useful in defining pulmonary fibrosis as the cause of shortness of breath in some patients. Roentgenographic evidence of pulmonary fibrosis may not necessarily indicate the extent to which it is a functional problem. Clinically, pulmonary fibrosis may be suspected in subjects without evidence of airway obstruction who reveal hyperventilation, clinically or by measurement, at rest and during exercise. The diagnosis may then be established by measuring a low diffusing capacity.

Summary

A simple method of estimating the diffusing capacity of the lung has been described in subjects without airway obstruction. The carbon monoxide uptake was measured and a value for alveolar carbon monoxide tension was estimated, based either on an assumed normal carbon dioxide tension in the arterial blood or, in persons with evidence of hyperventilation, on an assumed ratio of dead space to tidal volume of 40 per cent. The estimated diffusing capacity agreed well with that calculated from the directly measured arterial carbon dioxide tension. Some of the applications and limitations of this technique are discussed.

Sumario

Método Simplificado para el Cómputo de la Capacidad Difusora del Pulmón en Sujetos sin Oclusión de las Vías Aéreas

Se describe un método sencillo para calcular la capacidad difusora del pulmón en personas sin oclusión de las vías aéreas. Se midió para ello la toma de monóxido de carbono y se computó una cifra para la tensión alveolar del monóxido de carbono, basada ya en una supuesta tensión normal del bióxido de carbono en la sangre arterial o, en personas con signos de hiperventilación, en una supuesta proporción de espacio muerto por volumen corriente de 40 por ciento. La capacidad difusora computada convino con la calculada a base de la tensión arterial del bióxido de carbono medida directamente. Se discuten algunas de las aplicaciones y limitaciones de esta técnica.

Resume

Méthode simplifiée pour l'évaluation de la capacité de diffusion du poumon chez les individus sans obstruction des voies aériennes

Description d'une méthode simple permettant d'évaluer la capacité de diffusion du poumon chez les sujets ne présentant pas d'obstruction des voies aériennes. L'absorption du monoxyde de carbone a été mesurée et on a assigné une valeur à la tension du monoxyde de carbone alvéolaire en se basant sur une tension présumée normale du CO 2 dans le sang artériel ou, chez les individus où existent des signes d'hyperventilation, sur un rapport présumé: espace mort/volume de l'air courant égal à 40%. La capacité de diffusion selon l'évaluation était tout à fait comparable à celle qui était calculée par la détermination directe de la tension du CO 2 artériel. Certaines des applications et limitations de la technique sont discutées.

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A SIMPLE TEST OF MAXIMAL EXPIRATORY FLOW FOR DETECTING VENTILATORY OBSTRUCTION[1, 2]

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Introduction

Tests of functional capacity of the lung are conveniently made when the subject performs a maximal voluntary effort. Estimation of the mechanical behavior of the lungs and associated tissues may be based on (*1*) a measure of the volume of gas which they may be made to contain, and (*2*) a measure of the maximal rate with which the gas may be moved in or out. The vital capacity provides a satisfactory estimate of the maximal volume, but none of the various methods for estimating the maximal rate of gas flow has been widely accepted. A new and very simple instrument for measuring this property of the respiratory system is presented.

Review of Existing Methods

Ventilatory obstruction as used here refers to a condition common to three clinical syndromes: active bronchial asthma, hypertrophic or obstructive emphysema, and severe cases of chronic bronchitis. The symptom of dyspnea, which is common to these disorders, has been shown to depend upon the maximal volume of gas which can be moved per unit of time (1–3).

During inspiration there is a generalized expansion of the lung in which all air passages participate; hence there is a progressive decrease in the resistance to air flow due to the increasing diameter of the air passages. During expiration, the opposite holds, and a progressive increase in resistance to air flow occurs due to the diminution in the size of the bronchioles. For this reason among others, the attention of workers has been directed to the maximal performance during expiration (1–6).

The earliest test of ventilatory obstruction to become well established is the maximal breathing capacity (7), or what workers in Great Britain call the maximal ventilatory capacity. This test requires the subject to breathe at his maximal rate, i.e., at such a combination of frequency and depth that the volume of gas moved is a maximum for a period of twelve, fifteen, twenty, or thirty seconds, depending on the laboratory. The test is strenuous for the patient, cannot be repeated without a rest period, and requires meticulous technique. The accepted manner of interpretation of the test (8) requires the calculation of a predicted value based on a regression equation determined by age, sex, and body surface area. Wright (9), among others, has even recommended that each laboratory determine its own normal values so that the variability of technique may be mini-

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[2] This investigation was made with the assistance of a grant from the Committee on Research, Council on Pharmacy and Chemistry, American Medical Association.

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mized. Thus a test result may be low because of ventilatory obstruction, diminished capacity of the lungs, submaximal effort, weakness of respiratory muscles, or faulty technique of performance or interpretation.

Gaensler's (2) introduction of a device for simultaneously indicating the vital capacity and the volume expelled during the first second of expiration has greatly facilitated the detection of ventilatory obstruction. When the results are expressed as the percentage of the total capacity which has been expelled during the first second of expiration, there is very little variation which can be ascribed to sex, age, or body build, and these variables may be ignored (10). The relationship of this test to the maximal breathing capacity test is close ($r = +0.88$) (2). There are two disadvantages of the test. First, it is a ratio, and, second, its accuracy and interpretation depend upon the denominator (vital capacity) to some extent. Since Gaensler's basic unit is a spirometer with electronic attachments, it is not easily portable; it is also more expensive than the apparatus to be described.

Carpenter and associates (3) have used the same equipment as Gaensler but measure the volume expelled in 0.75 seconds as a way of indirectly estimating the maximal breathing capacity.

Leuallen and Fowler (6), using expiratory vital capacity curves obtained on a recording spirometer, graphically estimated the flow rate for the middle half of a maximally deep and forceful expiration. This period was selected because their spirometer had sufficient inertia to distort the early period of expiration. The reliability of the readings was low, with a significant difference in duplicate readings made from the same curve by different observers. The selection of a mid-period of expiration appears to be arbitrary, as does in fact the first second, or any other time. These writers called their test "maximum mid-expiratory flow," by which was meant flow rate during the middle half of expiration, following maximal inspiration, and when expiration is performed with maximal effort. In addition to these two uses of maximal, maximal expiratory flow is used here to describe the maximal value of flow during such a respiratory effort.

Observations

Because of these drawbacks in available tests for estimating the maximal rate of expiratory air flow, work was undertaken on a different type of instrument, based upon the measurement of flow itself rather than upon some combination of volume and time. Silverman and Whittenberger (11) have shown that, when air flows through a 400-mesh Monel wire screen, the pressure drop across the screen bears a linear relationship to the volume rate of air flow through the screen (within limits determined chiefly by the size of the screen). A suitably mounted screen called a pneumotachograph, when connected to a pressure transducer and recorder, inscribes a curve which can be calibrated to record air flow. The principle is illustrated in figure 1. An indicating instrument was designed using the same principle, but with the fragile wire screen replaced by a rugged porous vitrified, cup-shaped grinding wheel (12). The combination of vitreous resistor, mouth-piece holder, and indicating pressure gauge is called a "Puffmeter".[5]

The instrument is shown in figure 2. The range of the pressure gauge is zero to four inches of water. The subject is usually unaware of any sense of resistance to breathing through this resistor. The calibration curve of the device obtained

[5] The Puffmeter is available from J. J. Monaghan Co., 500 Alcott Avenue, Denver, Colorado.

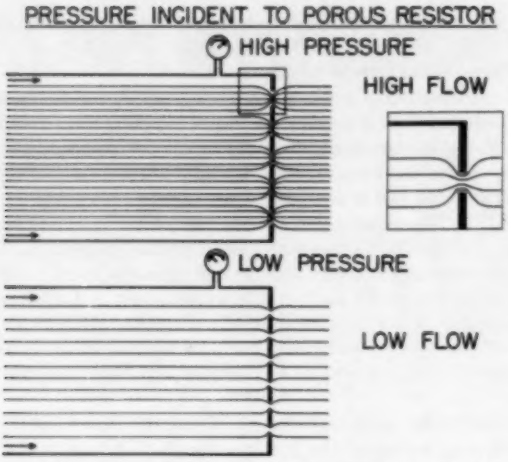

FIG. 1. Schematic illustration of the laminar flow resistor used as a flowmeter. The pressure due to the resistance is transmitted proximally from the pore which is shown in the inset.

against steady flows of air may be utilized as a meter face, thus permitting the direct reading of the meter in liters per minute. The performance of the test requires the subject to take in a full breath of air and to expel it as fast as possible through the flow resistor while the maximal deflection of the pressure gauge is noted. The maneuver is identical with that used for the timed vital capacity, except that the subject need not expel all the air he can, and the device has a single value to read. Because of the ease of the test, a number of repeated values may be obtained. When more than about 50 consecutive breaths are blown through the resistor, condensation of water leads to falsely high readings. The resistor may be dried by blowing compressed air or oxygen through it; under ordinary laboratory circumstances, this has been unnecessary as tests are usually at least twenty to thirty minutes apart and the resistor spontaneously dries in the air.

Gray and associates (7) pointed out the difficulty of determining which value of a series of presumed tests of maximal function to choose as the value to represent the test. Two factors affect the test reading: the variability of the test procedure and the coordination of all the physical and volitional factors which determine maximal performance. With the first, variability may occur in either direction; with the latter, by definition variability can lead only to falsely low values. Previous workers have used the maximal value for the vital capacity, the maximal breathing capacity, and the one-second vital capacity ratio but, since repetition of these tests is usually done only once or twice, little practical importance was attached to whether the mean or the maximal value was used. The Puffmeter test is capable of many replications in a short space of time without fatiguing the subject; this property of the test could be used to advantage in obtaining a more stable estimate of the individual's capacity.

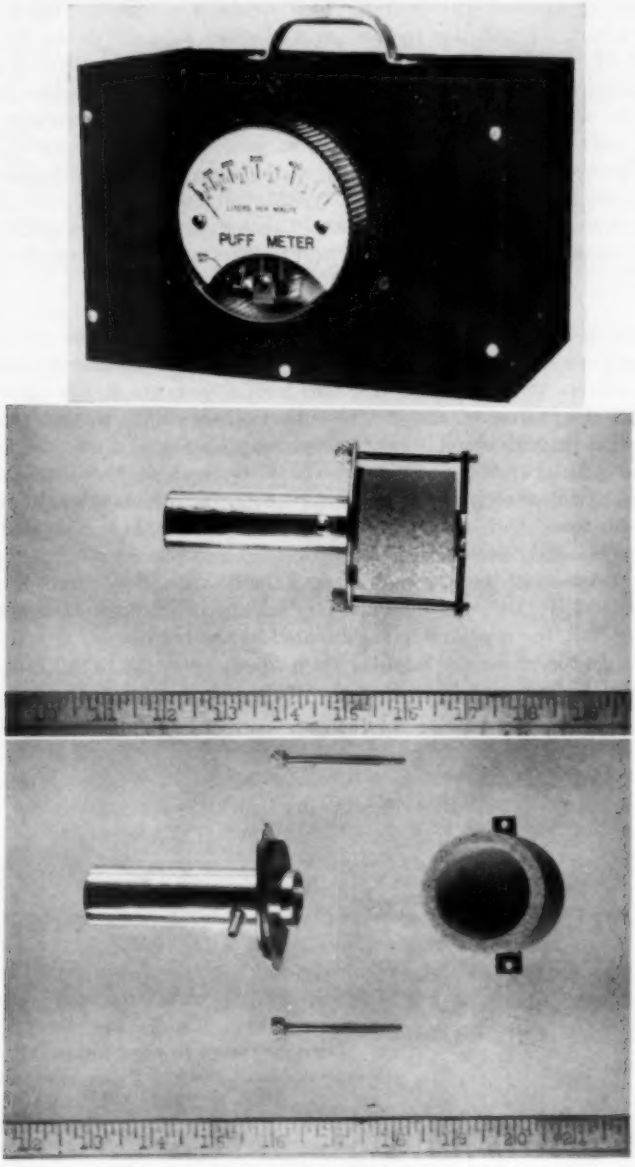

FIG. 2. The Puffmeter.

(Top) Showing the calibrated meter face.
(Center) The assembled resistor and mouthpiece holder.
(Bottom) The same exploded.

A sampling method was desired which would minimize the three possible types of error: (*1*) that due to random deviations inherent in the instrument and method; (*2*) that due to the subject's voluntary coordination of physical determinants of maximal performance; and (*3*) that inherent in the observer's reading the maximum of a transient needle deflection. It was arbitrarily decided that ten tests were to be taken for each subject and that *the mean of the three tests in sequence which had the highest sum* would be used to represent that patient. For example, of the ten test results: 250, 325, 280, 260, 270, *285*, *290*, *295*, 260, 300, the mean of the sequence 285, 290, 295 has the greatest sum of all the possible sequences; and the value of 290 would be given as the result on that subject.

Validity of the Puffmeter

A major question had to do with the relationship between the maximal flow value read from the Puffmeter and the true value of the maximal expiratory flow. To approximate the true value, a screen pneumotachograph was connected in series with the Puffmeter, and the pressure drop across the pneumotachograph was recorded through a strain-gauge amplifier on a Sanborn recorder (with the assistance of Dr. Jere Mead). On a series of thirty-one actual breaths in a normal subject who deliberately altered his performance, the relationship as depicted in figure 3 was found with a correlation coefficient of 0.992. It is clear that, within the range observed, there is a linear relationship between the maximal deflection of the Puffmeter and the maximal value of the recorded flow curve. It can thus be concluded that, although the Puffmeter reading is less than the true maximal expiratory flow, the relationship is estimated by the regression formula in figure 3. The range for which the relationship holds includes the useful range of the instrument. It is in this sense that the Puffmeter is proposed as a device for

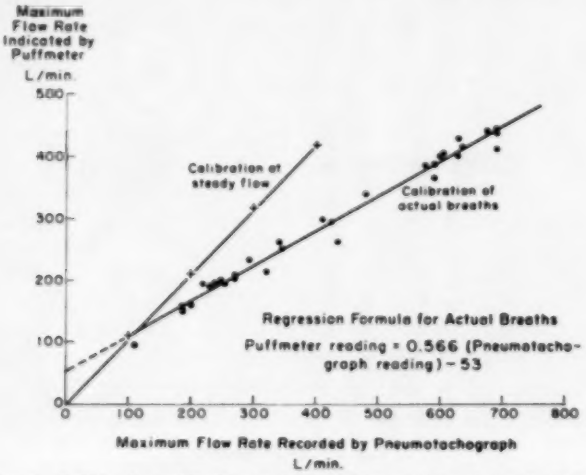

FIG. 3. Calibration of Puffmeter for actual breaths and steady flow.

TABLE 1

PUFFMETER MEANS OBTAINED AND ESTIMATES OF DAILY VARIABILITY BY SEX FOR A SAMPLE OF THREE MALE AND THREE FEMALE SUBJECTS

	Males	Females
Grand mean	419.6*	275.8
Residual standard error of mean of highest sequence of 3	18.3	9.0
Standard error due to day-to-day variability	29.6	11.7

* In this and subsequent tables, Puffmeter readings are based on a calibration at 37° C. For calibration at other temperatures, a correction factor of $1 + (0.006)\ (T - 37)$ is needed where T is temperature in degrees Centigrade.

estimating maximal expiratory flow. Subsequent data are based on the steady-flow calibration.

Since June, 1955, Puffmeter measurements were made on all subjects studied at the pulmonary physiology laboratory at Firlands Sanatorium, Seattle, Washington (with the cooperation of Dr. C. J. Martin, assisted by Miss Florence Best). Of 233 sets of data obtained through August, 1956, a correlation coefficient between the Puffmeter readings and the maximal breathing capacity test of plus 0.937 was observed. Furthermore, the coefficients of correlation and of regression were not significantly different for the last eleven months when compared with those of the first three.

Reliability of the Puffmeter

In the first experiment ten breaths were observed for 8 subjects on three days. These 240 tests were simultaneously and independently recorded by one experienced and one inexperienced observer. By the method of analysis of variance,[6] it was possible to compare the contributions to observed variation of (*1*) observer accuracy in reading the maximal needle deflection, (*2*) daily variation in performance of subjects, (*3*) different subjects, and (*4*) inherent variation of the method. The contribution of observer reading to the test variability was not significant.

The first experiment had been planned to give data for estimating the variance due to day-to-day differences; 7 of the subjects were female and one male, but differences by sex were suspected. A second experiment was carried out primarily to estimate daily variability separately by sex, and 3 male subjects and 3 female subjects were tested for four days each, this time by a single observer. The results are shown in table 1.

In the variance analysis, the contribution of day-to-day variability is not

[6] The total squared deviations from the mean were partitioned by standard methods (13) into fractions associated with: differences among subjects, between breaths, between observers, among days, and residual interactions. The variation between observers was less than the residual variation. The standard error associated with the observer differences was 10.4 liters per minute. The residual standard error of a mean of the highest sequence of three breaths was 14.9 liters per minute.

TABLE 2

TABLE OF PUFFMETER VALUES OBSERVED FOR THE NORMAL REFERENCE POPULATION (Age 40-65)

	Number	Mean	Standard Deviation	Coefficient of Variation
				per cent
Males	38	368.0	53.5	14.5
Females	41	293.0	43.8	15.0

TABLE 3

TABLE OF CORRELATIONS OF PUFFMETER READINGS WITH OTHER VARIABLES

	Number	Age	Height	Weight	Body Surface Area
Males	38	−0.13	+0.25	−0.07	+0.05
Females	41	−0.25	+0.46	−0.23	+0.58

statistically significant when tested against the residual variance by the variance ratio (F. ratio).

Variability between instruments is dependent upon uniformity of the flow resistor and of the indicating pressure gauge. The uniformity of the vitreous wheels is such that 20 of 22 tested had essentially identical characteristics. The accuracy of the gauge is guaranteed by the manufacturer to be within 0.05 inches of water. In the work reported here, data were based on individual instrument calibrations. Calibration is readily tested using a calibrated orifice and U-tube manometer, with a vacum cleaner as an air source.

Normal Test Values

The Puffmeter was used to estimate the maximal expiratory flow in a population sample.[7] From the first 340 persons studied, a "normal reference population" was selected by excluding those with any of the following conditions:

Previous history of pulmonary tuberculosis, pleurisy, hay fever, or asthma
Current history of dyspnea on exertion, of persistent cough, or recent pulmonary infection
Physical findings of chest deformity, rales, or evidence of transthoracic surgery, or
Roentgenographic evidence of pulmonary emphysema, fibrosis, or pleural deformity.

The results based on the mean of the highest sequence of three are given in table 2.

Correlations of the Puffmeter values with age, height, weight, and body surface area were examined, with the results shown in table 3.

Of this array, only the correlations for height and body surface area for females

[7] At the Heart Disease Epidemiology Program in Framingham, Massachusetts, a population sample has been under periodic clinical and laboratory evaluation since 1949. Vital capacity, one-second vital capacity ratio, and Puffmeter tests were performed beginning with the fifth biennial examination of this group of some 4,000 persons between forty and sixty-five years of age.

are significantly different from zero at the 1 per cent level. The remainder are not significant at the 5 per cent level. Since these two are by definition closely correlated themselves, only the greater, that due to body surface area, is considered further. The use of a regression formula based on body surface would reduce the standard deviation by about one-fifth or the coefficient of variation to about 12 per cent. Considering the difficulty in using a formula, this does not seem practicable. The data confirm the impression gained from the findings of others (7, 8, 10, 14) that there may exist sex differences in the determinants of normal values. This impression will be tested when a larger set of data becomes available.

The observations on the normal reference population were further analyzed by computing the means for the highest value observed in ten breaths and for the highest value observed in the first three breaths. The average values for the population and the standard deviation within the two sex groups were similar to comparable values based on the highest sequence of three as indicated in table 4.

In order to arrive at a value below which it may be said that the result would be unlikely to occur in a normal person, a point two standard deviation below the mean for the highest sequence of three breaths was calculated; assuming a statistically normal distribution, 2.3 per cent of the observations upon a normal reference universe would be expected to lie below this point. These values are shown in table 5.

TABLE 4

VALUES OBTAINED IN NORMAL REFERENCE POPULATION BY THREE METHODS OF SAMPLING BREATHS

	Number	Mean of Highest Sequence of Three	Highest Breath of Ten	Highest Breath of First Three
Males	38	368.0 ± 53.5	381.8 ± 53.7	364.2 ± 58.9
Females	41	293.0 ± 43.8	301.6 ± 41.4	283.2 ± 46.7

TABLE 5

VALUES BELOW WHICH THE PUFFMETER MAY BE SAID TO HAVE AN ABNORMAL VALUE

(Age 40–65)

Males	261
Females	205

TABLE 6

PER CENT INCREASE IN TESTS AFTER BRONCHODILATOR TREATMENT (N=43)

	Maximal Breathing Capacity	Vital Capacity	Puffmeter
	per cent	*per cent*	*per cent*
Mean	28.4	16.7	24.6
Standard error of mean	3.4	3.1	3.3

Comparative Sensitivity of the Test

In the population studied at Firlands Sanatorium, there were 43 occasions in 41 subjects in which a bronchodilator drug was used, and the maximal breathing capacity, vital capacity, and Puffmeter reading were taken both before and after the treatment. The per cent increase for each test is shown in table 6.

Discussion

Compared to the variability of the test within groups of normal persons, the variability due to daily variation and to observer reading is of little importance.

Needham and associates (10), after removing the regression due to age, height, weight, and body surface area, found for maximal breathing capacity a coefficient of variation of 14 and 16 per cent, respectively, for normal males and females; for 2-second expiratory volume, 11 and 14 per cent; and for vital capacity, 11 and 13 per cent. Without removing the effects of these variables, the values for the Puffmeter are quite similar. Pemberton and Flanagan (14) found the coefficient of variation for the one-second volume vital capacity ratio to be 9.1 per cent for males, which is less than the variability of the Puffmeter test. Tests of vital capacity and one-second volume vital capacity ratios for the normal reference population give results similar to these.

A "peak flow meter" has been developed in Great Britain by B. M. Wright, and its use in a population survey is reported by Higgins (15). The results with this instrument appear to be similar to those of the Puffmeter. The instrument is constructed on a different principle, namely that of a peak-indicating vane-type anenometer.

Since the Puffmeter test is very easily performed, requires no special skill or training for the operator, and has only a single value to read, it should be preferable to other methods for screening purposes in physicians' offices and in clinics. The fact that the subject can see directly the results of his performance diminishes the need for motivating him to maximal performance.

Summary

A simple method is described for obtaining an estimate of the maximal rate of air flow during expiration from the maximal inspiratory position. The test correlates closely with the maximal breathing capacity test and with the maximal rate of air flow of a recording pneumotachograph. The variability of the test is similar to that of the timed vital capacity and the maximal breathing capacity. It is substantially easier to perform than these tests, and the results may be read directly from the instrument. These characteristics make it suitable for clinical or screening use in the detection of persons with pulmonary air-flow obstruction.

Sumario

Sencilla Prueba de la Corriente Espiratoria Máxima para Descubrir la Oclusión de la Aireación

Descríbese aquí un método sencillo para obtener un cálculo de la velocidad máxima de la corriente de aire durante la espiración por medio de la posición aspiratoria máxima La.

prueba correlacionó íntimamente con la prueba de la capacidad respiratoria máxima y con la velocidad máxima de la corriente de aire de un neumotacógrafo registrador. La variabilidad de la prueba es semejante a la de la capacidad vital registrada y de la capacidad respiratoria máxima. Es decididamente más fácil de ejecutar que dichas pruebas y pueden leerse directamente los resultados en el instrumento. Estas características hacen que se preste para fines clínicos o de triaje en el descubrimiento de personas que padecen de oclusión pulmonar de la corriente de aire.

Resume

Test simple du débit expiratoire maximum pour déceler l'obstruction à la ventilation

Description d'une méthode simple permettant l'évaluation du débit d'air durant l'expiration à partir d'un mouvement inspiratoire maxima. Ce test correspondait étroitement au test de capacité respiratoire maxima et avec la vitesse maxima du débit de l'air enregistré sur le pneumotachographe. La variabilité de ce test est semblable à celle de la capacité vitale minutée et de la capacité respiratoire maxima. Cette méthode est substantiellement plus facile à pratiquer que ces tests et les résultats sont directement visibles sur l'instrument. Ces caractéristiques rendent ce test approprié à l'usage clinique ou au dépistage de l'obstruction au courant de l'air pulmonaire.

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PHYSIOLOGIC DEFECTS IN CHRONIC BRONCHITIS[1, 2, 3]

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INTRODUCTION

Chronic bronchitis, as a clinical entity without associated pulmonary disease, is usually of minor medical significance since it produces little or no disability. However, considered as a precursor to diffuse obstructive chronic pulmonary emphysema, it assumes more importance since the latter has become one of the common disabling diseases of the older age group. Since the etiology and natural history of chronic emphysema are unknown, and since it is usually in an advanced stage when diagnosed by clinical means, a prospective study of persons with chronic bronchitis may elucidate factors of significance in the development of emphysema. This report is concerned with the physiologic findings of the initial study of a group of subjects with chronic bronchitis who are to be restudied over a period of many years.

Clinical reports of chronic bronchitis have stressed the importance of bronchial infection and bronchial irritation in its causation. The high incidence of pneumococci and influenza bacilli in the bronchial secretions of such patients has been noted by a number of observers (1–3), but the exact role played by these microorganisms in this disease remains unsettled (4). Some workers (5–7) consider that industrial air pollution with bronchial irritants is of etiologic significance in chronic bronchitis. However, Phillips and his associates (8) found that the incidence of chronic cough did not correlate with industrial exposure, whereas it did show a significant correlation with the degree and duration of cigarette smoking. The possible importance of cigarette smoking in bronchitis has also been mentioned by others (9–12).

Although some investigators have measured various aspects of pulmonary function in chronic bronchitis, only Bates and his co-workers (13) performed studies in sufficient detail to separate such persons from those with emphysema. Although these writers did not specify what physiologic criteria they accepted as evidence for emphysema, they did find a mild reduction in vital capacity and "mixing efficiency" of the lung and a moderate to severe reduction in maximal

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ventilatory capacity in 14 subjects whom they considered to have chronic bronchitis.

It is evident from the literature that the term chronic bronchitis has often been used to describe conditions in which there is parenchymal lung disease in addition to bronchial abnormalities; and most frequently this associated pulmonary pathology is emphysema. In view of the reported disparity between the clinical diagnosis of emphysema by experienced chest physicians and its laboratory verification (14), it is obvious that, for purposes of study, the presence of emphysema can only be established by adequate laboratory investigation. From these considerations, it would seem important that both clinical and physiologic criteria be used to define chronic bronchitis.

For the purpose of this study, chronic bronchitis has been defined as a condition characterized by a cough which occurs at least ten times per day every day for at least six months in the absence of evidence of any other bronchopulmonary disease, including emphysema as determined by lung volume measurement. Productivity of the cough was not used as a distinguishing factor, and a paroxysm of coughs was considered as one cough for this definition. As determined by clinical and roentgenographic examination, the presence of bronchiectasis, pneumoconiosis, neoplasia, tuberculosis, or other inflammatory, fibrotic, or granulomatous disease of the lung (as well as pulmonary disease secondary to cardiac or other systemic illness) was a cause for exclusion from this study. A characteristic history of paroxysmal bronchial asthma was also a reason for exclusion unless it was of less than a year's duration in the distant past. If persistent spontaneous or post-tussive rales were heard in the chest, bronchographic examination was done to rule out the presence of bronchiectasis. Emphysema was considered to be present when the ratio of residual to total lung volume was greater than 35 per cent, together with a normal or greater than normal total lung capacity. Persons found to have emphysema by this definition will be followed as a separate group in this study.

Materials and Methods

Subjects for study: Thirty-eight hospitalized men and 6 male hospital employees whose evaluation fulfilled the above clinical criteria for chronic bronchitis were selected for study. Quotation marks for the word emphysema are used to indicate those 8 patients who were so classified after lung volume measurement. Twelve hospital employees who had no cough or other evidence of disease by history, physical examination, and chest roentgenogram served as a control group for the physiologic studies.

Methods of study: Clinical evaluation included a history of previous respiratory and allergic diseases, a list of all occupations and places of residence, and a detailed smoking history. Bacteriologic study of the sputum was done in some instances. Measurements of lung volume, maximal ventilatory capacity, index of intrapulmonary mixing, ventilation, gas exchange, and arterial oxygen saturation at rest and during and following a standard exercise test were made according to the methods described by Baldwin, Cournand, and Richards (15). The uniformity index was measured by the technique of Fowler (16). A further measure of intrapulmonary gas distribution was obtained by a continuous recording of the nitrogen concentration in the air expired forcefully at the end of a seven-minute period of oxygen breathing by means of a nitrogen meter and oscillograph. Airway resistance and pulmonary compliance were determined by the method of Mead and Whitten-

berger (17) with the modifications previously reported (18). The maximal diffusing capacity was measured according to the methods described by Riley and his co-workers (19).

Observations

Clinical Studies

The clinical observations are summarized in tables 1 and 2 and figures 1 and 2.

As judged by the duration of cough and the quantity and character of the sputum (figures 1, 2), this group represents a wide span of clinical severity of chronic bronchitis. In this respect it should be pointed out that only 3 of these patients entered the hospital because of their cough, the remainder having been found by a specific search for patients with a chronic cough. In a few instances, cough had not been noted on the initial hospital studies, either because the patient or the physician had not considered it to be abnormal. Two patients had histories characteristic of paroxysmal bronchial asthma of less than a year's duration, fourteen and ten years, respectively, prior to the time of study. None complained of dyspnea. On physical examination the only finding of note was the presence of inconstant rhonchi in 12 patients. One or more sputum cultures were performed in one-third of the group, and the only potential pathogenic organisms found in high incidence were Pneumococcus and *H. influenzae*. The majority of the group (table 1) had had pneumonia or pertussis in the distant past, but in only one instance did the chronic cough have its onset with the pulmonary infection. A history of more than one episode of respiratory infection per year which was associated with an increase in cough was elicited in 19 per cent, and some

TABLE 1

Chronic Bronchitis

Miscellaneous Clinical Characteristics of 36 Patients

	Per Cent
History of prior pneumonia	45
History of more than one acute bronchial infection per year	19
Upper respiratory tract disease (sinusitis, nasal polyps, et cetera)	14
Occupational exposure to bronchial irritants (judgment based on U. S. Department of Labor Bulletin #41)	30

TABLE 2

Chronic Bronchitis and Cigarette Smoking

Pack-Years	Number	Per Cent
Nonsmokers	5	15
<10	5	15
10–19	5	15
>20	18	55
Total	33	100

Pack years = number of packs per day × years.

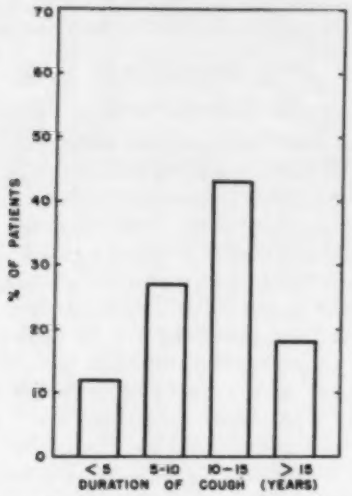

FIG. 1. Chronic bronchitis: duration of cough.

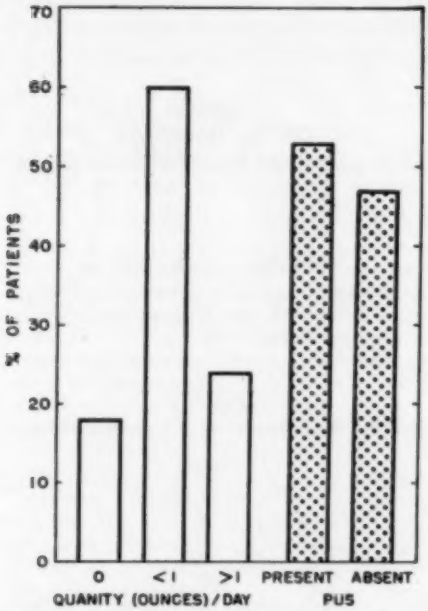

FIG. 2. Chronic bronchitis: quantity and character of sputum.

chronic disease of the upper respiratory tract was present in 14 per cent of this group. Although none of these patients could be considered to have a pneumoconiosis by chest roentgenography, almost one-third of them had an occupational exposure of one or more years to some form of bronchial irritant. The histories of cigarette smoking (table 2) in this group revealed that 15 per cent were nonsmokers and that 55 per cent had a cumulative smoking rate of greater than 20-pack years. (Pack years = number of packs per day × years.)

Of the 8 patients who were classified as having "emphysema" after pulmonary function tests had been completed, only 2 entered the hospital because of their cough. With respect to duration of cough, the quantity and character of sputum, incidence of pulmonary infection or asthma, and amount of cigarette smoking, no striking differences were apparent between this group and those with bronchitis. It may be significant, however, that all of the "emphysema" group were dyspneic on exertion and demonstrated rhonchi or wheezes in their chests.

Physiologic Studies

The results of physiologic studies are shown in tables 3 to 5 and figures 3 to 7. In comparison with data for the group of patients with chronic bronchitis, the data are shown for normal subjects and for that group of patients who, after physiologic study, were classified as having emphysema.

TABLE 3

INTRAPULMONARY GAS MIXING IN CHRONIC BRONCHITIS

	Lowest 7-Minute Alveolar Nitrogen	Uniformity Index	7-Minute Alveolar Nitrogen Concentration (Nitrogen Meter)
	per cent		
Normal	1.0–2.0	0.57–1.0	No rise
Chronic bronchitis			
Normal	19	6	2
Abnormal	17	4	4
Total	36	10	6
Per cent abnormal	47	40	67

TABLE 4

AIRWAY RESISTANCE AND PULMONARY COMPLIANCE IN CHRONIC BRONCHITIS

Number	Patient	Airway Resistance	Pulmonary Compliance
		cm. H_2O/liter/sec.	*liters/cm. H_2O*
	Normal	2.5 ± 1.29	0.30 ± 0.18
3	W. W.	4.5	0.22
5	M. H.	2.6	0.33
7	D. McD.	5.7	0.24
9	J. E.	5.1	0.21

TABLE 5

CHRONIC BRONCHITIS

Index of Intrapulmonary Mixing and Arterial Oxygen Saturation

	Arterial Oxygen Saturation (R or E)			Total	Per Cent Abnormal
	Normal	Abnormal	Not Done		
Lowest alveolar nitrogen per cent					
Normal	5	8	6	19	17/36 = 47%
Abnormal	5	9	3	17	
Total	10	17	9		
Per cent abnormal	17/27 = 63%				

Abnormal by either measurement = 25/36 = 69 per cent.
R = Rest E = Exercise

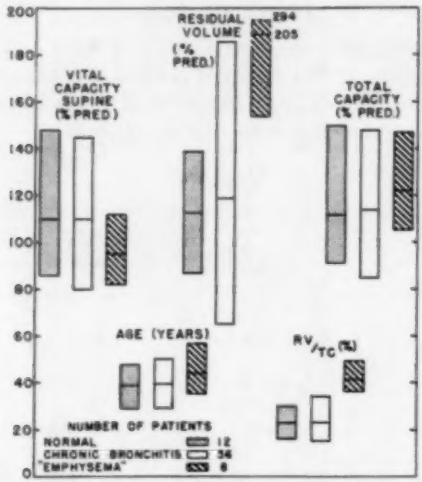

FIG. 3. Lung volume in normal subjects, chronic bronchitics, and "emphysema" patients Each bar represents range of values. Line within bar represents mean.

The range and mean values for age and lung volumes are illustrated in figure 3. No statistically significant differences between the means of age and the various lung volumes were demonstrated between the normal and chronic bronchitis groups. However, it is of interest that with respect to age and vital capacity—characteristics not used as a basis for separation of groups—the difference of the means between the "emphysema" group and the other two is significant ($P < 0.01$).

It is evident (figure 4) that most of the subjects with chronic bronchitis had normal maximal ventilatory capacities and, in fact, the difference between the

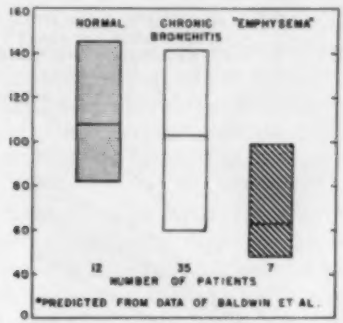

FIG. 4. Maximal ventilation capacity (per cent predicted*).

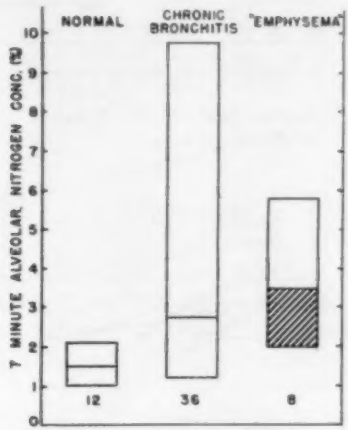

FIG. 5. Index of intrapulmonary mixing. *See* legend for figure 3. Number beneath each bar refers to number of patients.

means for these two groups is not significant. However, 8 such patients were below the limit of the normal ranges noted in this laboratory and by Baldwin, Cournand, and Richards. It is again noteworthy that the difference between the mean maximal ventilatory capacity of the chronic bronchitis group and that of the "emphysema" group is significant ($P < 0.01$).

In figure 5 the range of all values for the alveolar nitrogen percentages after seven minutes of oxygen breathing for each of the three groups are plotted. The difference between the means for the normal and chronic bronchitis groups is statistically significant ($P < 0.01$), but this is not true between the latter and the "emphysema" groups. In table 3 a comparison may be seen of three methods of evaluating the intrapulmonary distribution of inspired gas in the patients with chronic bronchitis. As judged by the lowest of duplicate alveolar nitrogen percentages from two oxygen-breathing periods by each patient, 17 of 36 (47 per

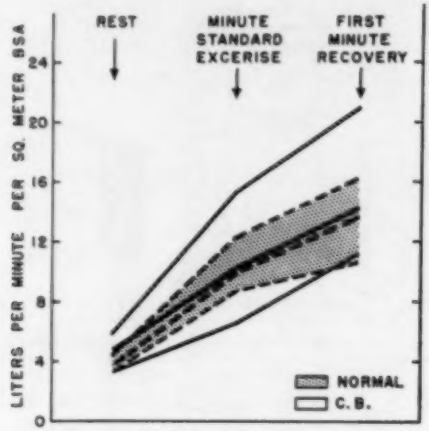

FIG. 6. Ventilation in chronic bronchitis.

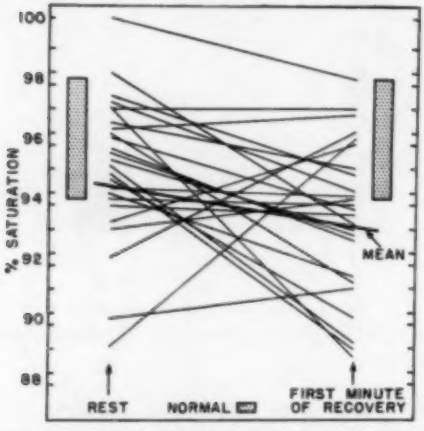

FIG. 7. Arterial oxygen saturation in chronic bronchitis. The values for per cent saturation at rest and in the first minute of recovery for each patient have been plotted and joined by a line. Omitted are patient *No. 2* (per cent saturation at rest—86; recovery—81) and patient *No. 18* (per cent saturation at rest—93).

cent) were abnormal. When 6 of the patients had, in addition, a measurement of the instantaneous nitrogen concentration by a nitrogen meter in a forced expiration after the seven minutes of oxygen inhalation, the results in 4 were abnormal. Finally, 4 of the 10 bronchitics had an abnormal uniformity index calculated from the single-breath test of Fowler and Comroe (16). As might be expected, the 4 subjects with abnormalities by the instantaneous nitrogen concentration analysis also showed abnormal findings by the usual method of

alveolar sample analysis. However, whereas the 4 subjects with abnormal uniformity indices also had abnormal values by the seven-minute test, 4 patients with normal uniformity indices had abnormal values by the seven-minute test.

A wider range of values for ventilation (figure 6) than normal was found in the chronic bronchitis group both at rest and during and after the standard exercise test, but no significant difference between the means for the two groups was demonstrated.

Although the mean arterial oxygen saturation (figure 7) was normal at rest and only slightly reduced after exercise in the chronic bronchitis group, it may be seen that a considerable number of these patients had arterial unsaturation in one state or the other. In the 9 patients in whom it was measured, the maximal diffusing capacity of the lung for oxygen was within normal limits, whereas the percentage of venous admixture during the steady-state exercise was elevated in 3 of the 9 patients.

Of the 4 subjects (table 4) in whom studies of the mechanics of respiration were carried out, 3 had increases in airway resistance whereas all had normal pulmonary compliance. Of interest is the fact that the seven-minute alveolar nitrogen was elevated in the 3 whose airway resistance was increased and was normal in the other individual. The maximal ventilatory capacity was normal in all 4 patients.

Discussion

It is evident from the observations reported here that some persons with chronic bronchitis, as herein defined, have mild but definite abnormalities of pulmonary function. With respect to the possible future development of emphysema in some of these patients, the abnormal alveolar nitrogen after seven minutes of oxygen breathing in 17 of 36 (47 per cent) of the group (table 5) is of particular interest since it is a defect so characteristic of this disease. This measurement is influenced by the volume of alveolar gas as well as by the distribution of inspired air, but the data in this study indicate that intrapulmonary gas distribution was at fault in those persons with abnormal values. Although the uniformity index calculated from the single-breath test confirmed this defect in function in 4 subjects, in 4 others this index was normal while the lowest of the seven-minute tests was abnormal. This suggests that the single-breath analysis is not always a more sensitive one for uneven alveolar ventilation than the seven-minute test. In addition, in judging abnormality on the basis of the lowest alveolar nitrogen obtained from two or three successive seven-minute runs, it seems likely that there has been a tendency to reduce the number of persons in this group whose inspired air distribution might be considered defective.

The arterial oxygen saturation (table 5) was reduced either at rest or following the standard exercise test in 17 of 27 (63 per cent) of the patients with bronchitis in whom it was measured. The relatively small degree of hypoxia and the normal maximal oxygen diffusing capacity of the lung in some of these patients indicate that the hypoxia does not arise from a reduction in the diffusing surface of the lung. That generalized alveolar hypoventilation could be responsible for the

hypoxia also seems unlikely since over-all ventilation was normal or elevated for the great majority of the group; and in the 9 patients whose gas exchange was studied in detail, alveolar ventilation was normal. Thus, the hypoxia observed probably arises in areas of the lung that are underventilated in relation to their circulation. The finding of an increase in the percentage venous admixture during steady-state exercise in only a few of those patients who had arterial hypoxia following an unsteady state type of exercise suggests that the latter is a more sensitive means for detecting abnormalities of alveolar ventilation-perfusion relationships.

When judged by the presence of either a high seven-minute alveolar nitrogen or a decrease in arterial oxygen saturation, it may be seen (table 5) that 25 of the 36 (69 per cent) of the patients with chronic bronchitis showed abnormalities. The lack of correlation between these two abnormalities seen in table 5 has also been observed in other diseases of the chest and attributed to various degrees of blood perfusion of regions of the lung that may or may not be poorly aerated (20). The data in table 5 also suggest that the measurement of arterial oxygen saturation is a more sensitive means of detecting localized alveolar hypoventilation than the measurement of the seven-minute alveolar nitrogen concentration.

The two principal physiologic defects observed, namely abnormal intrapulmonary gas distribution and arterial hypoxia, most likely arise from localized areas of poor aeration in the lung which in turn could result from either bronchiolar obstruction or regional alteration in the elastic properties of the lung. Although the mechanics of respiration were studied in only 4 patients, the increase in airway resistance in 3 and the normal pulmonary compliance in all 4 suggest that bronchiolar obstruction is the more likely explanation for the gas exchange defects. Further, the normal maximal ventilatory capacity in most of these patients suggests that this bronchiolar obstruction exists in relatively few bronchioles.

Since an increase in the residual volume of the lung has been observed in normal subjects more than fifty years old (21), it is evident that this finding alone cannot serve to define the presence of diffuse obstructive emphysema. In this study, the finding of a significantly decreased vital capacity and maximal ventilatory capacity in the "emphysema" group indicates that an increase in the residual lung volume in these subjects is simply another physiologic expression of their airway obstruction. These abnormalities allow the conclusion that the "emphysema" group demonstrates more extensive or more severe bronchial obstruction than the bronchitic group. However, neither the physiologic nor the clinical observations allow a qualitative differentiation of the two groups.

Summary

Forty-four patients considered to have chronic bronchitis have been subjected to physiologic study. For the purpose of the study, chronic bronchitis has been defined as a condition characterized by a cough occurring at least ten times per day, every day for at least six months, in the absence of evidence of any other bronchopulmonary disease. After pulmonary function testing, 8 of the patients

were considered to have chronic pulmonary emphysema. The physiologic data on the remaining 36 patients indicate that persons with chronic bronchitis as herein defined frequently have abnormal intrapulmonary distribution of inspired air and/or a disproportion between alveolar ventilation and perfusion in some parts of their lungs. These defects give rise to mild abnormalities in oxygen transfer. The findings have been interpreted as evidence of bronchiolar obstruction in this group.

It seems likely, in view of the type and incidence of functional abnormalities, that some of these patients with chronic bronchitis are in an early phase of the natural history of chronic pulmonary emphysema.

Sumario

Defectos Fisiológicos en la Bronquitis Crónica

Cuarenta y cuatro enfermos que pasaban por tener bronquitis crónica fueron objeto de un estudio fisiológico. Para los fines del estudio, se definió la bronquitis crónica como dolencia caracterizada por tos que se presenta a lo menos diez veces al día, cada día a lo menos seis meses, sin haber signos de ninguna otra afección broncopulmonar. Después de comprobar la función pulmonar, se consideró que 8 de los enfermos tenían enfisema pulmonar crónico. Los datos fisiológicos para los 36 enfermos restantes indican que las personas que tienen bronquitis crónica en la forma aquí definida muestran distribución intrapulmonar anómala del aire aspirado y/o desproporción entre la ventilación y la perfusión alveolares en algunas partes de los pulmones. Estos defectos producen leves anomalías en el intercambio de oxígeno. Interprétanse estos hallazgos como signos de oclusión bronquiolar en este grupo.

A la luz de la forma e incidencia de las anomalías funcionales, parece probable que algunos de estos enfermos de bronquitis crónica se encuentran en una fase incipiente de la historia natural del enfisema pulmonar crónico.

Resume

Effets physiologiques dans la bronchite chronique

Quarante-quatre malades, que l'on a considéré comme étant atteints de bronchite chronique, ont été soumis à une étude physiologique. Pour les besoins de l'étude, la bronchite chronique est définie comme une affection caractérisée par une toux survenant au moins dix fois par jour, chaque jour pendant au moins six mois, en l'absence de signes de toute autre affection broncho-pulmonaire. A la suite de l'épreuve de la fonction pulmonaire, 8 patients ont été considérés atteints d'emphysème pulmonaire chronqiue. Les données physiologiques obtenues chez les 36 autres malades indiquent que les sujets atteints de bronchite chronique, selon la définition précitée, présentent fréquemment une répartition anormale intrapulmonaire de l'air inspiré où une disproportion entre la ventilation et la perfusion alvéolaires dans certains segments du poumon, où ces deux anomalies. Ces altérations fonctionnelles sont responsables de légères anomalies dans les échanges d'oxygène. Dans ce groupe, les resultats ont été interprétés comme des signes d'obstruction alvéolaire. Etant donné le type et l'incidence des anomalies fonctionnelles, il semble admissible que certains de ces malades atteints de bronchite chronique présentent la phase de début de l'histoire naturelle de l'emphysème pulmonaire chronique.

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IMMUNITY IN EXPERIMENTAL TUBERCULOSIS[1]

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INTRODUCTION

It is well known that in tuberculosis a primary infection affords a certain amount of resistance to reinfection. Such processes of immunity have, in general, been studied in animals, using two main criteria, i.e., increase in survival time of vaccinated animals after infection with a dose of bacilli sufficient to kill all of the unvaccinated controls in a certain time; and the difference in the number of living bacilli recovered from the organs of vaccinated and normal animals infected with a small dose of virulent bacilli (1).

In such studies it is not possible to follow the course of the development of the disease, either after primary inoculation or after reinoculation, without sacrificing the animal. Such experiments, moreover, involve the use of a large number of animals, and the results usually have to be assessed statistically.

A new method has recently been described to follow the development of immunity in mice (2, 3). This involves immunizing the animal with an intracorneal or intradermal injection of *M. tuberculosis* and challenging the animals by a subsequent intracorneal inoculation (in the second eye if the first eye has been used for the immunizing injection). When an animal is infected intracorneally for the first time, a lesion develops in the cornea which increases in size until it occupies most of the cornea. In a previously immunized animal, however, no lesion develops in the infected cornea at all after challenge, provided the immunity is adequate. This method has been used to follow quantitatively the development of immunity in mice under a variety of experimental conditions.

It has been demonstrated by Swedberg (4) that the production and development of immunity in animals varies considerably with the species of animal used and the strain and dose of organisms used for vaccination and challenge. It is thus very difficult—or sometimes even impossible—to compare the results of different workers in the field of immunity to tuberculosis.

In an attempt to overcome this difficulty, the present writers have performed an extensive series of investigations on the development of immunity, using one strain of mouse kept under controlled conditions and, for most of the work, one strain of *M. tuberculosis*. It is thus possible to compare directly many of the results obtained in different experiments performed under these same conditions. The results will be described under three headings, namely, (*1*) the effect of the time interval between the immunizing and challenging doses on the development of immunity; (*2*) the effects of varying the sizes of the immunizing and challenging doses; and (*3*) the production of immunity by bacilli killed by different methods.

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Material and Methods

The mice used throughout were a mixed albino strain which has been bred in this laboratory for the past ten years. At the start of the experiments the mice were about three months old, weighing 20 to 25 gm. The average life span of these mice is two to three years. The primary inoculations (vaccination) were made with the following material:

A virulent strain of *M. tuberculosis* var. *bovis* (originally supplied by Dr. Levinthal) which has been maintained in Dubos medium as specified by the Medical Research Council (1948) and passaged through mice after every seven subcultures to maintain and confirm its virulence. Dilutions for inoculations were made in normal saline containing 0.05 per cent Tween 80®, and were made up fresh on each day as required. These were prepared from the second to the seventh subcultures after seven to thirteen days of incubation.

Cultures of *M. tuberculosis* var. *bovis* killed by different methods as described below.

Routes of primary inoculation

Intracorneal: This was carried out by the technique described by Rees and Robson (5) but using ether anesthesia alone.

Intradermal: The mice were lightly anesthetized with ether. The hair over the flank was removed by plucking, and the required amount of the material was injected intradermally in the flank with a micrometer syringe. With the size of inoculum used for vaccination with the live virulent strain, none of the animals died of systemic disease during the many months of observation following vaccination.

Intravenous: The required amount of the material was injected into a tail vein, using a tuberculin syringe.

Reinoculation (challenge)

All of the mice were reinoculated in the cornea with the required dilution of the same virulent strain of *M. tuberculosis* var. *bovis* as was used for the vaccinations, and a control group of mice was kept in all instances for the challenge dose.

The pathogenicity of the strain of tubercle bacilli used for reinoculation in the present study has been tested on many occasions in this laboratory, and corneal lesions have consistently been produced in mice in a dilution of 10^{-3}, i.e., about 500 organisms, and, when a homogeneous suspension is used, in a dilution of 10^{-4}. The volume inoculated was always about 0.01 ml.

Assessment of results: The immunity induced in the mice was measured by one or more of three criteria: percentage of vaccinated animals developing lesions in the challenged eye; difference in the size of the corneal lesions in vaccinated and control animals (this method only was used in mice in which the vaccination was done by the intradermal route, since, for humane reasons, it was not desired to produce lesions in both eyes of any one animal); and estimation of the relative number of bacilli in the corneas of vaccinated and control animals by staining of the whole cornea. This was done by the method described by Robson and Sullivan (6*a*) which gives an evaluation of the number of bacilli present by comparison with an arbitrary scale (*T* scale).

Results

The Rate of Development and the Duration of Immunity Following a Primary Intradermal Inoculation with a Virulent Strain

A number of mice were inoculated intradermally into the flank with 0.01 ml. of a 10^{-3} dilution of a culture of virulent *M. tuberculosis* var. *bovis*. A group of 6 such vaccinated mice were reinoculated in one cornea with 0.01 ml. of the same dilution and strain of organisms at intervals of zero, one, two, three, four, five, seven, and fifteen days and one,

three, and six months. A control of 6 nonimmunized mice was kept for each group of the reinoculated mice. All of these animals were observed for a period of fifteen to twenty-eight weeks after reinfection. All but one of the control mice in all groups developed corneal lesions after the usual incubation period of ten to fourteen days. These lesions increased in size and reached a maximum after another fortnight, regressed somewhat, and then remained stationary for the remainder of the observation period.

The immunity in the groups challenged at the various periods has been calculated as the percentage of animals developing lesions at the site of reinoculation at any time after challenge. The results are shown in figure 1. It will be seen that immunity developed rapidly during the first week after vaccination and reached 100 per cent when the interval between inoculations was seven days, when none of the challenged animals developed any macroscopic lesions. This degree of immunity was seen thereafter when the interval was increased to six months between vaccination and challenge. Another group of animals which had been inoculated in one cornea with the same size of inoculum was similarly challenged thirteen months later in the second cornea, and none of the animals developed any lesions in the challenged eye, having thus maintained immunity up to this period. The lesions which developed in the animals challenged respectively one and two days after vaccination were smaller than those in the appropriate control animals, all of the lesions in these groups being in fact smaller than the average for the controls.

In figure 2 is shown the number of animals which had lesions at various times after challenge of the groups challenged during the first week after vaccination (only 5 animals were challenged at two-day intervals). It may be seen that lesions which appeared in animals inoculated when some immunity was present occasionally resolved, leaving a macroscopically clear cornea, an event not seen in lesions from a primary inoculation. The lesions which resolved completely were in all cases small at the stage when they were macroscopically visible. The slight regression in lesion size which occurs in most animals after about one month was probably sufficient to account for the complete disappearance of these tiny lesions in partially immune animals.

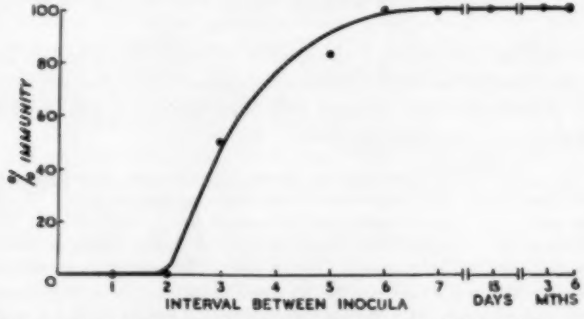

FIG. 1. Showing the development of immunity in mice challenged intracorneally at various periods after intradermal immunization. Immunity expressed as percentage of challenged eyes which did not develop any macroscopic lesion.

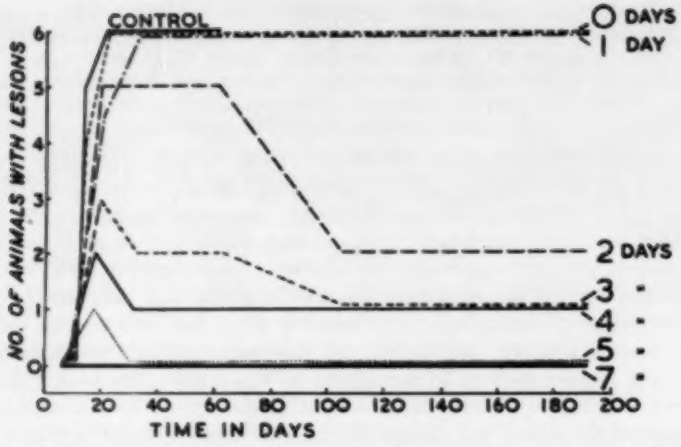

FIG. 2. Data based on same experiment as in figure 1. The curves show the number of eyes with macroscopic lesions in the groups challenged at various periods after immunization (from one to seven days) and at various periods after challenge. Note that some macroscopic lesions actually disappeared. The total number of animals in each group was 6, except for the two-day interval when it was 5.

Seven months after vaccination all of the mice of the groups challenged respectively five days and one month after vaccination were killed and the corneas were removed. These corneas were stained, and the extent of the bacillary mass was determined on the *T* scale. All of the corneas of the vaccinated group challenged after a five-day interval showed acid-fast bacilli in one or two places in compact masses, the remainder of the cornea being free of acid-fast bacilli. The *T* values of these eyes ranged from *T* 2 to *T* 5. In animals reinoculated after a one-month interval, no acid-fast bacilli were found in 2 corneas, and 3 showed only a few degenerated acid-fast bacilli at a level of much less than *T* 1. The significance of these results will be dealt with in the discussion.

In order to determine whether there were any live bacilli in the immune eyes after they had been subjected to a prolonged period of immunity in the animals, two groups of animals were treated with cortisone, as it has been shown that cortisone will reactivate a chronic lesion (6b).

In one of the groups the interval between the immunizing and challenging dose was seven days (3 mice) and in the other the interval was three months (6 mice). The cortisone administration was started thirty weeks and seventeen weeks, respectively, after challenge and was continued for eleven weeks. The dose was 0.5 mg. of cortisone acetate daily for six days a week, given subcutaneously. One animal of the second group died at the end of eleven weeks of cortisone therapy, and the experiment was then terminated. The other animals were then killed and the corneas were removed and stained for acid-fast bacilli. Massive systemic tuberculosis was found in the animal that had died. Small tuberculous lung lesions were also found in one of 3 mice of the first group and in 3 of the 5 surviving mice in the second group.

In one mouse of the first group small cords of bacilli were found in the cornea, showing reactivation of the lesion with multiplication of bacilli under cortisone therapy. In none of the other animals, either of the first or second group, was there any evidence of bacillary multiplication and, in fact, no bacilli were found in any of the corneas of the second group. With one exception, therefore, these experiments gave no evidence of the presence of live bacilli in the immunized corneas. The systemic lesions of these animals probably arose from the bacilli of the immunizing infection, which was given intradermally.

Comment: From these results it may be seen that the use of a challenge inoculum of about 500 viable units provides a sensitive indicator for the detection of the development of immunity in mice. Thus, fairly small differences in resistance can be measured and the actual rate of increase in resistance in animals subsequent to vaccination can be followed. This probably depends on the fact that a very small intracorneal inoculation will produce a lesion with a high degree of reliability. In a total of 193 nonimmunized mice infected by one investigator with this size of inoculum as controls, a corneal lesion failed to develop in only one animal. Thus, in a group of 6 animals, the probability of one not developing a lesion is 0.03 and the probability of two not developing lesions is 0.0004. Consequently, if in any group 2 animals fail to develop lesions due to prior vaccination, it may be stated that a significant increase in resistance has occurred ($P = 0.0004$). In this experiment, 3 animals in a group of 6, challenged three days after vaccination, failed to develop lesions, so that by the third day a highly significant increase in resistance had occurred ($P = 0.000{,}003$).

In these experiments the percentage immunity has been calculated as percentage of animals in each group in which no lesions developed after intracorneal inoculation. If no lesion developed in any of the animals in a group, the immunity has been called 100 per cent. This does not imply that the immunity is in fact complete and that no bacterial multiplication occurs, but merely that under the conditions of the experiments the particular inoculum used for challenge does not produce a macroscopic lesion.

The results presented in figure 1 show that vaccination with 0.01 ml. of 10^{-3} dilution of virulent *M. tuberculosis* var. *bovis* produced 100 per cent immunity (as defined above) to reinoculation with the same size of inoculum in all the groups of mice challenged, when the interval between vaccination and challenge was seven days or more. No immunity could be demonstrated during the first two days after vaccination, but between the second and seventh days there was a rapid and regular increase in the immunity. Once the immunity had reached a maximum, no decrease was observed up to an interval of thirteen months between the inoculations.

It is, however, possible to have quite appreciable bacillary multiplication without the production of a macroscopic lesion. It may be that, although there is 100 per cent immunity by seven days after vaccination, as judged by the appearance of a macroscopic lesion in the challenged eye, there may nevertheless still not be enough immunity at this stage to inhibit bacillary multiplication completely. The results of the staining of the corneas of animals challenged

five days and one month after vaccination showed that there had been considerable multiplication in the former case, but there was no evidence that any multiplication had occurred in the latter case. It may be seen in figure 2 that only one animal in the group challenged after five days developed a lesion, but considerable multiplication of bacilli was shown to have occurred in all of the animals of this group. It may be assumed, therefore, that there was also multiplication of bacilli in some other animals which showed 100 per cent immunity as judged by the previous criteria. When the animals were challenged one month after vaccination, it was observed that no multiplication had occurred at all. Thus, complete immunity, sufficient to inhibit completely multiplication of this dose of bacilli, was present at least when the interval between vaccination and challenge was one month.

Although no such detailed experiments have been described previously, Bloch and Segal (7) reported that, in mice immunized with either BCG or a large dose of heat-killed bacilli, immunity could be detected by a challenge given three days after immunization. They also reported that when BCG was used for immunization, the immunity reached a maximum after three weeks and had regressed completely after nine months. Dubos and Pierce (8), however, showed that, when mice were immunized with even a very low dose of BCG, immunity could still be detected when the experiments were terminated one year after immunization. These results are in general agreement with those obtained in the present experiments, and the minor differences are probably due to variation in strains of animals, the bacilli used, and the method of assessing immunity.

There is another point which deserves discussion in connection with the development of immunity. It has hitherto been tacitly assumed that the restricted evolution of the lesions, produced by a microbial challenge made a few days after vaccination, represents the influence of that vaccination on that challenge infection from the early moments of its inception. Such an assumption, however, is not necessarily true. It may well be that, when an intracorneal (or an intradermal) dose is given to a nonimmunized animal, immunity only starts developing approximately seven to ten days later, and then gradually increases. This would account for the self-limiting nature of the corneal lesion, since the increasing immunity would bring the multiplication of the organisms to an end, usually at a level around T 7. When the challenging dose is given some two to three days after immunization, the immunity due to the first inoculum will become effective before the lesion has appeared in the challenged eye, and thus have time to depress the rate of development of such a lesion as compared with eyes in control animals in which no vaccinating dose was given.

The Effect on Immunity of Varying the Immunizing and Challenging Doses

In order to determine how the size of the immunizing dose affects the degree of immunity produced, groups of mice were inoculated intracorneally with two sizes of inocula of a culture of *M. tuberculosis*. These were then challenged at

intervals with three different doses of a culture of the same organisms. The details are as follows:

Two groups of 72 mice were inoculated intracorneally with an undiluted culture and a 10^{-3} dilution of a culture of *M. tuberculosis* var. *bovis*, respectively. Both of these groups were subdivided into twelve subgroups of 6 animals, and each subgroup was reinoculated in the other cornea with either a 10^{-1}, 10^{-2}, or 10^{-3} dilution of the same strain of organisms at intervals of one, two, four, or eight weeks. A control group of 6 nonvaccinated mice was kept for each subgroup of the reinoculated mice.

Assessment of results: By the second week, all of the nonvaccinated mice inoculated with 10^{-1}, 10^{-2}, and 10^{-3} dilutions developed corneal lesions which reached a maximum after another two weeks, regressed slightly, and then remained stationary for the remaining period of observation.

The vaccinated groups were observed for a period of nine to twenty-five weeks for the development of any corneal lesions. Since the writers did not wish to produce a developing lesion in both eyes of any one animal, mice showing a lesion in the challenged eye were killed. The percentages of the animals that developed a corneal lesion in the challenged eye in the different groups at any time during the period of observation are shown in table 1.

It will be seen first of all that, when immunity is produced in mice, challenge with a small inoculum produces no lesion, whereas a larger inoculum breaks down the immunity and leads to the production of macroscopic lesions. This was seen in the animals vaccinated with both an undiluted and a 10^{-3} dilution of the culture. The possible reasons for the breakdown with a larger inoculum will be discussed later. Second, these results show that immunity becomes progressively more effective up to four weeks after inoculation. Again this was seen in both groups: In those vaccinated with an undiluted culture and challenged with a 10^{-1} dilution, the percentage of animals which developed lesions

TABLE 1

SHOWING THE DEVELOPMENT OF IMMUNITY AT VARIOUS STAGES AFTER VACCINATION, USING A VARIETY OF CHALLENGING DOSES

Vaccination	Interval Between Inocula	Per Cent of Animals Developing Lesions with Different Challenge Doses			Period of Observation
		10^{-1} Dilution	10^{-2} Dilution	10^{-3} Dilution	
	weeks				*weeks*
Undiluted culture	1	100 (5)	0 (5)	0 (6)	25
	2	50 (6)	0 (5)	0 (5)	24
	4	32 (6)	0 (6)	0 (6)	22
	8	14 (7)	0 (6)	0 (6)	18
10^{-3} dilution of the culture	1	100 (6)	80 (5)	40 (5)	16
	2	80 (5)	16 (6)	20 (5)	15
	4	32 (6)	16 (6)	0 (6)	9
	8	0 (6)	0 (5)	0 (6)	9

Figures in parentheses show number of animals.

progressively fell from 100 to 14 per cent when the interval between the two injections was increased from one to eight weeks. Likewise, with the 10^{-3} immunizing dose (and 10^{-1} challenge), the percentage of animals which developed lesions fell from 100 to 0 per cent over the same time interval.

There did not appear to be any great difference in the final degree of immunity conferred upon the animals by the two immunizing doses (undiluted and 10^{-3} dilutions). At one and two weeks after inoculation, some animals immunized with the 10^{-3} dilution developed lesions when challenged with dilutions up to 10^{-3}, whereas with the larger immunizing dose only a challenge with the largest inoculum (10^{-1}) evoked a macroscopic response. This suggested that at this early date the immunity was somewhat greater with the larger vaccinating inoculum. Later on (at four and eight weeks), however, there was no significant difference between the two groups.

A second experiment was performed, however, to test this further. The range of vaccinating doses was extended while the challenging doses were so chosen as to be likely to overcome the immunity partially and so produce lesions, and were therefore high (undiluted and 10^{-1} dilutions). The time of challenge was fixed at four weeks, by which time the full immunizing effect was expected. The details were as follows:

Six groups of 14 mice were vaccinated intracorneally with 0.01 ml. of a 10^{0}, 10^{-1}, 10^{-2}, 10^{-3}, or 10^{-4} dilution and intravenously with 0.1 ml. of an undiluted culture of *M. tuberculosis* var. *bovis*. These groups were divided into two subgroups of 7 animals each and were challenged four weeks later in the other cornea with either an undiluted or 10^{-1} dilution of the same strain of organisms. A group of 7 nonvaccinated mice were included as controls for each of the challenge doses. The animals were observed for a period of eight weeks.

Assessment of results: The primary inoculation given intracorneally produced lesions in all of the mice. In the controls for the reinoculation, all animals developed corneal lesions. Lesions also developed in many of the challenged eyes and, since the intent was not to produce a developing lesion in both eyes of any one animal, the mice showing such lesions were killed. The percentages of such animals developing a corneal lesion in the different groups at any time during the period of observation are shown in table 2.

This second experiment again consistently showed a higher occurrence of lesions with the larger challenging inoculum (undiluted as compared with 10^{-1} dilution). The results also suggest that the smallest immunizing dose confers more immunity than the larger immunizing doses. Thus, in all of the groups immunized with the undiluted culture, 10^{-1}, 10^{-2}, and 10^{-3} dilutions intracorneally, or with the undiluted culture intravenously, some of the animals challenged with the 10^{-1} dilution developed corneal lesions, whereas none did so when the immunizing dose consisted of a 10^{-4} dilution and the challenging dose was a 10^{-1} dilution.

The results of the experiments show that the effect of varying the immunizing and challenging doses must be considered separately since there is at first sight no obvious correlation between them.

Effects of varying the immunizing dose: From table 1 it appears that the larger

TABLE 2

SHOWING THE EFFECT OF TWO DIFFERENT CHALLENGE DOSES IN ANIMALS IMMUNIZED WITH A VARIETY OF INOCULA

Challenge	Per Cent of Animals Developing Lesions in Groups Previously Immunized with:						Nonvaccinated Controls
	Intravenous Undiluted 0.1 Ml.	Intracorneal Undiluted	10^{-1}	10^{-2}	10^{-3}	10^{-4}	
0.01 ml. undiluted.....	67 (6)	80 (6)	83 (6)	57 (7)	86 (7)	100 (7)	100 (7)
0.01 ml. 10^{-1} dilution.....	50 (6)	56 (7)	25 (4)	33 (6)	20 (5)	0 (6)	100 (7)

Figures in parentheses show number of animals.
Interval between inocula—4 weeks.
Period of observation—8 weeks.

immunizing dose gave better protection against the challenge than the smaller dose, as more animals developed lesions after challenge in the latter group. In table 2, however, the opposite trend is seen, and better immunity was obtained from the smaller vaccinating doses. The numbers of animals used in these groups were too small to allow any statistical analysis to be made of the results, but it would seem that in any case the effect of varying the immunizing dose is not very striking and disappears with time after vaccination.

Effect of varying the challenging dose: The result of varying the challenging dose is much more obvious, and it may be clearly seen from the results of both experiments that, in assessing the degree of immunity present in animals, the results obtained may depend to a large extent on the size of the challenging dose used. Thus, some animals which show complete immunity to challenge with 0.01 ml. of a 10^{-3} dilution of a culture containing about 500 organisms will show no immunity or relatively little immunity when the challenging dose is increased a thousand fold, i.e., 500,000 organisms. This is of particular importance when investigating different levels of immunity produced by various experimental conditions. In the second experiment (table 2), when the immunity produced in animals immunized by various doses was being tested, if the immunity produced had been tested only by the use of a large challenging dose, no difference between the groups would have been seen. Similarly, in the first experiment (table 1), animals in various groups showed complete immunity to a small challenge inoculum, but when the inoculum was increased systematic differences in animals were disclosed. Thus, it is obviously important when examining for immunity that various sizes of the challenging dose should be used so that fine differences in immunity may be observed.

This problem has been discussed by Youmans and Youmans (9), using intravenous challenge in mice, who also showed that the results obtained in immunity experiments could vary depending on the size of the challenge used. Thus, if the size of the challenge dose were outside certain limits, the presence of immunity could be masked by other factors, depending on the normal response of the mice to a heavy or mild infection.

One further conclusion which can be drawn from the results of the first experiment is that for any size of immunizing dose the quantity of immunity present in the animal will increase with time after vaccination. In the group of animals immunized with the 10^{-3} inoculum, none showed immunity to challenge with undiluted culture when the interval between vaccination and challenge was one week. When the interval between the inoculations, however, was increased, there was a progressive increase in immunity until the interval between inoculations was eight weeks, by which time all of the animals showed complete immunity.

These results are in agreement with those of Swedberg (4) who studied the immunity produced by BCG in white mice. He could find no systematic difference in the immunity produced when the vaccinating dose was varied between 0.05 mg. and 3.2 mg. of bacilli. He did, however, show that the immunity obtained increased with the interval between vaccination and challenge to a limit of about thirty days, and furthermore that the survival time increased with the decreasing size of the challenge dose. If the challenge dose were increased sufficiently, then it completely overwhelmed the immunity. He could find no difference in the immunity produced when the vaccine was given by either the intravenous or intraperitoneal route, but both of these routes gave significantly more immunity than the subcutaneous route.

The effect of varying the immunizing dose of BCG vaccine in guinea pigs is reported in the Tuberculosis Program of the United States Public Health Service (10). It is stated that a progressive increase in immunity was obtained with increasing doses of vaccine ranging from 1:100 to ten times the dose used for human vaccination.

Detailed Study of the Breakdown of Immunity with a Large Challenge Dose

It has been shown previously that, when immunity is produced in mice and the cornea then challenged with a small dose of tubercle bacilli, not only does no macroscopic lesion develop, but no multiplication of the bacilli occurs. Hence, for long periods after challenge only a few bacilli, corresponding to the number inoculated at the challenge, can be found (3). In the present experiments, however, it has been demonstrated that, when the challenge dose is much larger, the immunity is overcome and macroscopic lesions do appear. It seemed advisable to study this difference further. It might be possible, for example, that, with the larger challenge, an insufficient number of phagocytes can migrate into the cornea to take up all of the bacilli. On the assumption that cellular factors are largely concerned in immunity to tuberculosis, the bacilli left extracellularly would then multiply, unchecked by the influence of immunity. This possibility was studied in the following experiment.

A number of mice were vaccinated by the intracorneal inoculation of a 10^{-3} dilution of a culture of *M. tuberculosis* var. *bovis*. After an interval of two weeks, to allow immunity to develop, these animals were divided into two groups which were challenged into the second cornea with a 10^{-1} and a 10^{-3} dilution, respectively, of a culture of the organisms. As controls, a similar number of nonvaccinated animals were inoculated with the same two

dilutions of the culture. On the day of challenge, and daily thereafter for seven days, 4 of the animals in the group challenged with the 10^{-1} dilution were killed and the challenged corneas were removed. At the same time the same number of the nonvaccinated controls were sacrificed and the corneas removed. In the group challenged with the 10^{-3} dilution, 2 animals were killed at the times shown in figure 3 and the corneas were removed. The corneas were examined by phase contrast microscopy and also by gross staining to assess the number of organisms present by determination of values on the *T* scale. From the values so obtained the multiplication of organisms in the various groups was determined.

Phase contrast examination of the "immune" eyes challenged with the 10^{-1} inoculum showed a number of bacilli in the first three days, mostly intracellular within macrophages, but some extracellular. On the third day a certain amount of cellular debris was already seen, however, suggesting bacillary multiplication, with the breakdown of cells. Extracellular bacilli were still seen at this stage, and this picture persisted until the appearance of a macroscopic lesion on the seventh day. In the nonimmune eyes, the phase appearances were qualitatively similar although the accumulation of cells and debris was more rapid, and a macroscopic lesion developed rather earlier. At the various stages observed in the immune and nonimmune eyes, the relative proportion of intra- and extracellular bacilli seemed essentially the same. It is clear, therefore, that the breakdown of the immunity with the 10^{-1} inoculum involved overgrowth of macrophages and was not due to extracellular multiplication of the bacilli alone.

The group of animals challenged with the 10^{-3} dilution and the unvaccinated controls challenged with the same inoculum behaved as previously described (3, 6*b*). With the primary inoculation, progressive multiplication up to a *T* 7 level by the fourteenth day was observed, whereas in the immune eyes no bacterial multiplication occurred. In the immune eyes, it is very difficult to estimate the relative number of intra- and extracellular bacilli at the various inter-

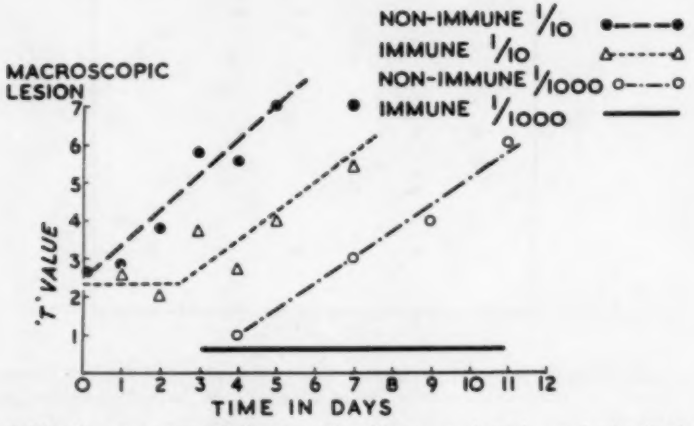

FIG. 3. Showing average multiplication of bacilli in control and immunized animals challenged two weeks after vaccination with two sizes of inocula, i.e., 1:10 and 1:1,000 dilution of a culture of *M. tuberculosis*.

vals after challenge, since, with the small number of bacilli present, it is impossible to locate many of them by phase contrast microscopy, and it is completely impossible to find them in normal histologic sections.

The averaged results of the bacterial multiplication in the various groups are shown in figure 3. In the two control (nonimmunized) groups, the bacilli multiplied progressively from the time of inoculation onwards and as previously described by Robson and Sullivan (6*b*), reaching a value of *T* 7 at a time which corresponded with the appearance of a macroscopic lesion. In the immune group, challenged with the 10^{-3} dilution of the culture, no multiplication was seen at all during the course of the experiment. The results of the group challenged with the 10^{-1} dilution seemed to show a delay in multiplication behind their controls, followed by multiplication at the same rate as the controls. That this may be a somewhat erroneous conclusion, however, may be seen when the individual *T* values obtained on each day are plotted, instead of the average of these values. This was done for both the groups challenged with the 10^{-1} dilution, and the result is shown in figure 4. From this it may be seen that various degrees of inhibition of the rate of multiplication of the bacilli were observed in the immune eyes. This ranged from little or no inhibition in some animals to complete inhibition of multiplication in others. It is noteworthy that in two immune eyes in which a level of *T* 5 and *T* 5.5, respectively, was observed, there were small macroscopic lesions (on the fifth day after inoculation);

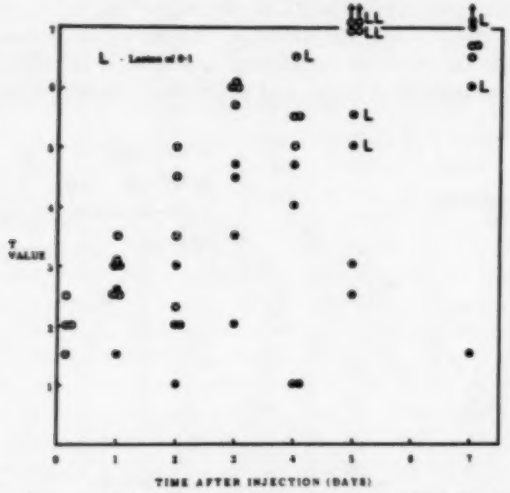

FIG. 4. Based on the same data as figure 3 showing the individual *T* values (as a measure of the number of bacilli in the cornea) in the different eyes. Animals challenged with the 1:10 dilution.

○—control mice
●—immunized mice
L—shows presence of macroscopic lesion

whereas normally, i.e., in nonimmune animals, macroscopic lesions do not usually appear below a bacillary level of *T* 7. These results demonstrate that the immunity produced by 0.01 ml. of a 10^{-3} dilution of a culture of organisms, when the immunity has been allowed to develop for two weeks, can be overcome by a challenge dose of 10^{-1} dilution, but not by a challenge with a 10^{-3} dilution of a culture.

A second experiment was performed in which the interval between vaccination and challenge was extended to four weeks in order to determine whether any greater degree of immunity would be observed, as would indeed be expected from the results described before.

Mice were immunized by the intracorneal inoculation of a 10^{-3} dilution of *M. tuberculosis* var. *bovis*. Four weeks later they were challenged in the second eye with a 10^{-1} dilution of a culture of the same organism. At the same time nonimmunized mice were also inoculated to serve as controls. Animals of both groups were killed at daily intervals, and the number of organisms in the cornea was estimated on the *T* scale. One cornea from each group was also examined each day by conventional histologic methods. The numbers of animals killed on each day and the *T* values of the corneas are shown in figure 5.

It will be seen that the controls behaved substantially as in the first experiment (figure 4). In the immune eye the degree of inhibition of bacillary multiplication again varied a good deal. In some animals there was complete inhibition, and no bacillary multiplication occurred. In others there was only a partial

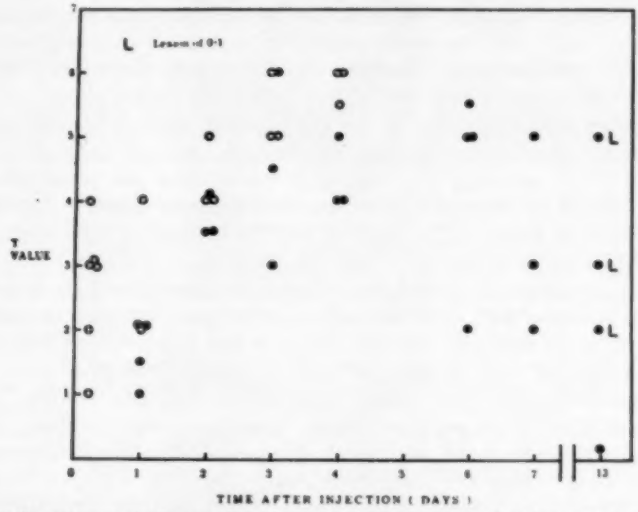

FIG. 5. Experiment similar to that shown in figure 4 except that interval between immunization and challenge was four weeks.

○—control mice

●—immunized mice

L—shows presence of macroscopic lesion

inhibition and, in fact, the highest T value observed in the whole group was only T 6, and this in only one animal. Observations were continued in this experiment up to the thirteenth day after challenge and the four eyes stained after this period had T values ranging from T 5 to less than T 1, showing that some inhibition of multiplication had occurred in all of these eyes. It is again noteworthy, however, that 3 animals had small macroscopic lesions although the T values of these stained corneas were respectively 5, 3, and 2.

Histologic examination: Careful histologic examination was performed on corneas inoculated with the 10^{-1} inoculum, both in immune and nonimmune animals. Serial sections were cut of corneas up to four days after inoculation in the nonimmune animals and up to five days in the immune animals. Alternate strips were stained with hemotoxylin and eosin and with either carbol fuchsin or nile blue sulfate.

The results are in close agreement with those previously reported (11, 12). There was no very great difference in the histologic picture between the two groups i.e., the nonimmune and immune animals. On the first day after inoculation (or reinoculation) there was heavy polymorphonuclear-neutrophilic invasion. This was rapidly succeeded by invasion by macrophages which quickly penetrated the neutrophilic mass so that by the second and third days after inoculation or reinoculation, many macrophages were found among the polymorphonuclear mass of cells. Thereafter the macrophages gradually supplanted the polymorphonuclears.

Careful examination of the limbus at the various stages was also informative. On the first day the limbus was occupied by a mass of polymorphonuclears, but later on this mass was replaced by macrophages so that by the third and fourth days the invading cells were almost exclusively macrophages.

The various sections stained by the Ziehl-Neelsen method or with nile blue sulfate were carefully searched for mycobacteria but, in spite of intensive examination and restaining of a number of the sections, practically none were found. It should be noted that there was no difficulty in demonstrating these organisms by the gross staining method used for estimating organisms in comparison with the T scale (6*a*, *b*). It was calculated that on the fourth day after inoculation, a section 7μ in thickness through the lesion would contain several thousands of organisms. It is thus all the more puzzling that no organisms were found in the sections, although this in is fact in agreement with the observations made by other workers following inoculation into other tissues (7).

When figures 4 and 5 are compared, it will be seen that there was less multiplication of organisms in the animals challenged one month after vaccination than in those challenged two weeks after vaccination, so that, in the former case, few animals showed multiplication to a level greater than T 5.

It was thought before embarking on this experiment that the actual mechanism of the "breaking through" of the immunity would be observed. For example, perhaps in the immunized group there would be increased extracellular multiplication or perhaps insufficient cell invasion to give complete immunity. The results, however, both of phase contrast and histologic examination, showed

that neither of these factors could account for the breakdown. Intracellular and extracellular multiplication was seen in the immunized animals in the same way and to the same extent, as far as could be judged, as in nonvaccinated animals. Thus it would appear that the overcoming of immunity by a large challenge dose is dependent on some factor which is not revealed by microscopic examination and is quite possibly of a biochemical nature.

The Development of Immunity Following Inoculation with Killed Tubercle Bacilli

There is a good deal of evidence that killed tubercle bacilli, or even extracts from such bacilli, can produce immunity, and this was investigated quantitatively with the present method.

Robson, Sullivan, and Didcock (3) have already reported that, when mice are inoculated intracorneally with an undiluted culture of the bovine strain killed by autoclaving, no immunity can be demonstrated to a subsequent challenge of the second cornea with a 10^{-3} dilution of the bovine culture. This was confirmed and then extended to experiments involving immunization with bacilli killed by other methods.

Cultures of *M. tuberculosis* var. *bovis* in Dubos medium were centrifuged, the medium removed, and the organisms resuspended in the same volume of 0.05 per cent Tween saline. These suspensions of organisms were then killed by the following methods:

The tubes were sealed and autoclaved at 30-pound pressure for twenty minutes.

The tubes were immersed in a water bath at 70°C. for forty-five minutes i.e., the method used by Bloch and Segal (13), and for ninety minutes.

The bacilli were suspended in ethyl alcohol as follows (as suggested to us by Professor G. A. H. Buttle): To the bacilli suspended in Tween saline, ethyl alcohol was added to give a final concentration of 70 per cent alcohol. The tubes were kept at 4°C. for seven days and after this period the bacilli were centrifuged, the supernatant removed, and the bacilli were finally resuspended in Tween saline to the original volume.

The bacilli were treated with phenol as follows: Phenol was added to the Tween saline-suspended bacilli to give a final concentration of 2 per cent phenol (1). The suspensions of bacilli so treated were kept at 37°C. for five, eight, and twenty-four hours, with occasional manual shaking. After these periods of incubation, the bacilli were removed by centrifuging and were resuspended twice in normal saline to remove the phenol; and finally the organisms were suspended in the original volume of Tween saline.

After all of these treatments the organisms were found to be clumped, and fine suspensions suitable for inoculation were made by thoroughly shaking with glass beads. The beads were then allowed to settle and the supernatant was used for inoculation.

The final suspensions of bacilli killed by heating in a water bath, ethyl alcohol, or phenol, were tested for sterility as follows:

Three sets of Dubos fluid medium were inoculated with 0.5 ml. each of the final suspensions and incubated at 37°C. for three weeks. No growth of the organisms was observed in any of the tubes.

Three sets of semi-solid agar medium (14) were inoculated with a drop of the final suspensions delivered by a Pasteur pipette and were incubated at 37°C. Final readings were taken at the end of four weeks. No growth was observed in any of these tubes.

A set of two Löwenstein-Jensen slopes were inoculated with a loopful of each of

the final suspensions of bacilli and incubated at 37°C. for four weeks. A few small colonies were seen growing on the slopes inoculated with suspensions killed in the water bath for forty-five minutes and ninety minutes. Acid-fast bacilli were found on microscopy of these cultures. No growth was found in any of the others.

Three mice were infected intravenously with 0.5 ml. of each of the final suspensions. One mouse was sacrificed after eleven weeks and the other 2 after twenty-one weeks, and the organs were examined macroscopically and microscopically. No macroscopic lesions were detected in any of these mice. A few acid-fast bacilli were found in smears from either lungs, livers, or spleens of animals in all of these groups, even after twenty-one weeks. The few acid-fast bacilli found in these smears examined up to twenty-one weeks (in the consistent absence of macroscopic lesions) were considered to be remnants of the original inoculum, since it has been shown that killed organisms will survive for several months in animals.

The immunizing properties of the organisms killed by these various methods were tested by inoculating them into groups of 5 to 7 mice. In all cases, 0.01 ml. of the undiluted suspension was given intradermally as the immunizing dose.

Two weeks later each animal was challenged by the intracorneal injection of 0.01 ml. of a 10^{-3} dilution of *M. tuberculosis* var. *bovis*. The development of lesions in the various groups is shown in figure 6. It may be seen that there was some immunity in all of these groups, and this was most striking in the animals immunized with organisms treated with phenol for eight and twenty-four hours.

A second experiment was then performed involving the use of larger doses of killed bacilli for immunization.

Groups of 4 to 7 mice were vaccinated by the intradermal or intravenous injection of organisms kept in contact with 2 per cent phenol for eight and twenty-four hours in the same way as before. One group of animals was inoculated with 0.1 ml. of the undiluted suspension and another group was similarly inoculated with ten times this dose (0.1 ml. of a tenfold concentration of the organisms). The animals were challenged after two weeks with 0.01 ml. of a 10^{-3} dilution of *M. tuberculosis* intracorneally.

The group vaccinated with the lower dose of killed organisms treated with phenol for 24 hours had a high mortality at reinoculation due to the anesthetic, and this group was discarded. The results in the other groups are shown in figure 7. It will be seen that in all cases there was definite immunity which was quite

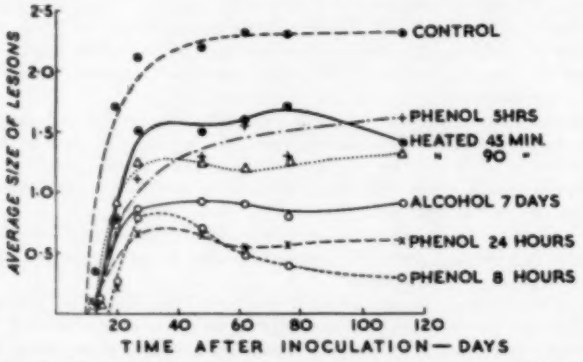

FIG. 6. Average size of lesion in animals immunized intradermally with bacilli killed by various methods and challenged after two weeks intracorneally.

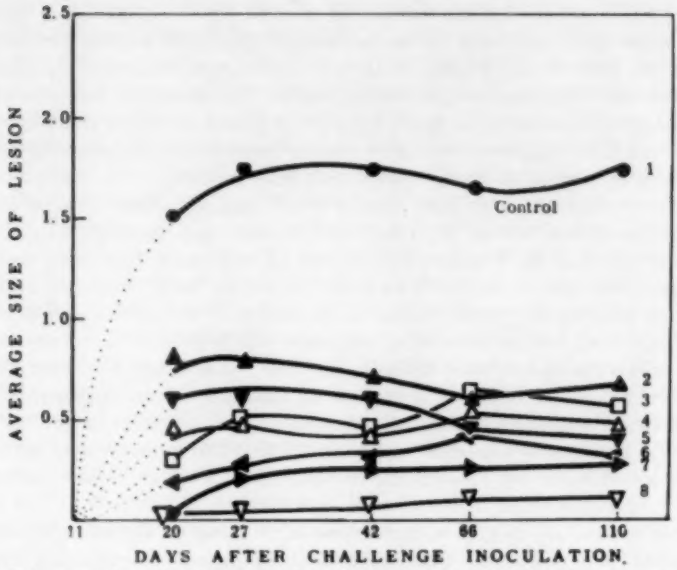

FIG. 7. Average size of lesion in animals immunized with bacilli killed by various methods and challenged after two weeks intracorneally:

1—Unvaccinated controls.
2—Undiluted culture killed by phenol for 8 hours, injected intravenously.
4—Undiluted culture killed by phenol for 24 hours, injected intravenously.
6—Undiluted culture killed by phenol for 8 hours, injected intradermally.
3—Culture concentrated 10 times killed by phenol for 24 hours, injected intravenously.
5—Culture concentrated 10 times killed by phenol for 8 hours, injected intravenously.
7—Culture concentrated 10 times killed by phenol for 8 hours, injected intradermally.
8—Culture concentrated 10 times killed by phenol for 24 hours, injected intradermally.

marked and sometimes almost of the order seen in animals vaccinated with live bacilli. The less effective route of immunization was that involving intravenous injection. These killed suspensions were not examined for the presence of live organisms, except for the tenfold concentration of the final suspension killed by 2 per cent phenol for twenty-four hours. One-tenth milliliter of this was inoculated subcutaneously in the groin of a guinea pig. The animal was killed seventeen weeks later and examined. No macroscopic lesions were detected in either lungs, livers, or spleens, and no acid-fast bacilli were found in smears of these organs.

Comment: In the experiments performed using killed bacilli for vaccination, the results show that the immunity produced depended on at least two factors. These were the size of the immunizing dose and the manner in which the bacilli were killed. When the bacilli were killed by autoclaving, no immunity was produced even when the dose used was 0.01 ml. of undiluted suspension (which was found to be about 3 γ dry weight of bacilli). When the organisms were

killed by the action of either alcohol or phenol, however, this dose of bacilli did produce some immunity. When the dose of the bacilli was further increased to 30 γ and 300 γ of bacilli killed by the action of phenol, an increase in immunity was observed with increasing size of inoculum. No difference was observed in the immunogenic activity of bacilli killed with phenol for either eight or twenty-four hours. This suggests that there was no degradation of immunogenic substance by the action of phenol within this time interval.

These experiments, therefore, clearly show that, although the smaller doses of killed bacilli are inferior to living bacilli in their immunogenic activity, with the larger doses of bacilli killed with phenol an immunity of the kind seen with live organisms can be obtained. It might be argued that the better immunity obtained with the live bacilli is due to the fact that, after multiplication at the site of injection, a larger number of organisms will be present than were injected in the killed vaccine. This is unlikely, however, since it has been found that a dose of about 3 γ of H37Ra will produce an immunity, even though this strain, which was investigated at the time, showed no multiplication in the cornea of mice (3). The results also show quite clearly that the immunogenic activity of bacilli, killed either by autoclaving or heating in a water bath, is inferior to that of organisms killed either by phenol or alcohol.

These results are in general agreement with those of Dubos, Schaefer, and Pierce (15) who also stated that bacilli killed by phenol were effective immunologically, whereas a heat-killed vaccine was not. Bloch and Segal (13), on the other hand, demonstrated immunity following vaccination with bacilli killed either by heat or by phenol. Swedberg (4), however, has shown that the immunogenic activity of a heat-killed vaccine may depend on many factors which are often overlooked. For example, he showed that the immunogenic activity of tubercle bacilli could vary, depending on the pH of the medium in which the bacilli are suspended during heat killing and also on the strain of bacillus used.

General Comment

These experiments show that, when mice are inoculated intracorneally or intradermally with *M. tuberculosis*, immunity develops very rapidly, so that some degree of immunity can be demonstrated when the challenge is given twenty-four to forty-eight hours after the primary inoculation. The immunity does not, however, reach its maximum until about one month after vaccination and remains for at least thirteen months, the maximal period of follow-up study. It should be noted that immunity was assessed on two main criteria, i.e., the appearance and size of macroscopic lesions in the cornea and the multiplication of the inoculum originally deposited within the cornea. These are in fact merely two aspects of a continuum. The organisms multiply and induce a cellular reaction; they also spread somewhat within the cornea. The maximal density they reach, is, however, not much above the *T* 7 level and the spread does not add very substantially to the total number of organisms within the cornea. At some stage of this multiplication, usually when a concentration equivalent to about a *T* 7 level has been reached, a macroscopic lesion visible under the binocular microscope appears.

The spread of the lesion to involve most of the cornea is not, as a rule, accompanied by any great increase in the total number of organisms present within the cornea. Hence, a decrease in the size of macroscopic lesions in immune animals—or even its complete suppression—as compared with nonimmune controls need not involve more than some inhibition in the multiplication of organisms. The decrease will, therefore, represent a very sensitive index for the effects of even a small degree of immunity. It is, therefore, not surprising that in the present investigation some degree of immunity could be demonstrated with a challenge given within a very short period of vaccination. Its further development, as observed with the present technique, to reach a maximum at about four weeks, when no bacillary multiplication at all occurs in the immune eye inoculated with about 500 organisms, is in agreement with previous findings.

Swedberg (4), using survival time as an index of immunity in mice, found that this was striking when the challenge was given thirty-five days after vaccination, but much less consistent at intervals of twenty-four and thirteen days. Martin (16) records that, when the interval between the protecting and challenging infection was varied between four and thirty days, a marked degree of resistance had developed when the interval was sixteen days or more. Bloch and Segal (13), inducing immunity with BCG, found that it reached its highest level between three weeks and three months after vaccination. These writers also observed that at six months the immunity had significantly decreased, and at nine months practically no immunity persisted. With the more sensitive method of the present experiments, an immunity sufficient to prevent the development of macroscopic lesions was observed up to thirteen months after vaccination. Unfortunately, these corneas were not stained so that it was not possible to compare accurately the immunity observed at that stage with the immunity present when it had reached its maximum.

The very rapid development of some degree of immunity (with a challenge given within twenty-four to forty-eight hours of vaccination) observed in the present experiments is difficult to explain in terms of the classical concepts of immunity. It must be remembered that the effect of such early immunity is actually observed only about a fortnight later, when macroscopic lesions develop which are smaller than those in control nonimmunized animals. It seemed possible that such an effect might be nonspecific in nature. For example, intradermal inoculation of foreign material might produce vascular reactions which themselves would affect the subsequent development of a lesion in another part of the body. Corneas of mice have in fact been injected with a variety of organisms, *B. coli*, *P. pyocyaneus*, and Pneumococcus, and two to three weeks later the other cornea has been challenged with *M. tuberculosis* to test for nonspecific effects. There was no evidence of any such increase in resistance to tuberculous infection. It was also impossible to demonstrate any immunity from the subcutaneous injection of an oil-in-water emulsion, as described by Jespersen and Magnusson (17).

The question also arises: How does an inoculum introduced into the cornea produce immunity when it is clear that the organisms remain strictly localized within the cornea, at least for several weeks after inoculation? There are two main possibilities, namely: that the immunity is actually produced in the corneal

tissue, and that an immunogenic substance (or substances) leaves the cornea and elicits the immunity elsewhere in the body.

With regard to the first possibility, there is evidence that antibodies can be produced in the cornea (18) and, if immunity in tuberculosis were indeed humoral, this would be the likely explanation. In fact, there is good evidence that cellular factors are involved in such immunity, and all of the present writers' observations make it highly unlikely that cells leave the vaccinated cornea to produce immunity, as seen by subsequent challenge of the second cornea. Unless it is assumed that immune bodies produced in the vaccinated cornea are subsequently taken up by cells in which they exert their immune function, there is left only the possibility that the immunity observed is due to the escape of immunogenic substance(s) from the cornea.

The possible mechanism by which the large inocula introduced into the cornea of previously immunized mice can overcome the immunity, and so multiply to produce lesions, also needs consideration. It is clear that this is not due to a failure of sufficient cells to invade the cornea, since large numbers of organisms were taken up by massive cellular invasion. Macrophages filled with bacilli were seen by phase examination as was cellular debris, suggesting that overgrowth of macrophages had occurred. Since practically no organisms could be found in the sections of the corneas, unfortunately no confirmation of these results could be obtained by the conventional histologic means. Hence, histologic examination gives no clue to the reason why the larger inoculum can overcome the immunity, whereas the smaller inoculum does not.

Immunologic paralysis following the injection of a large dose of antigen has been described by Felton (19) and is discussed by Burnet (20). Such a large dose of antigen will not only prevent the development of immune antibodies following the subsequent administration of a small dose of antigen, but will also destroy the immunity produced by the previous administration of a small dose of antigen. This is indeed in essence the phenomenon that is seen in the cornea which is inoculated with a large number of tubercle bacilli: the immunity which is effective in preventing the multiplication of a small number of organisms fails to meet the challenge of a larger inoculum. Immunologic paralysis has been attributed to the adsorption of antibody by the large store of antigen, and this will thus explain a paralysis of an immunity dependent on antibody production. This would explain the phenomenon seen in the cornea if immunity in tuberculosis were due to the formation of antibodies, which is, however, unlikely. Although the mechanism of immunity in tuberculosis is not clear, there is much evidence which implicates monocytes as responsible for inhibiting the multiplication of organisms in immune animals. On such an hypothesis the observations of the present writers could be explained as follows: When a small inoculum is put into the cornea, only a few organisms are taken up by each invading macrophage and thus there is enough "antibody-like" substance in each macrophage to deal with the bacilli. With a larger inoculum, however, the "antibody-like" substance in the macrophage is swamped and the non-neutralized bacilli are able to multiply and overgrow the cell. It is realized that the concept advanced is highly

theoretical and makes a number of assumptions, but it does bring the phenomenon observed in tuberculosis in line with that described by Felton and subsequent workers. It also serves as a basis for additional experimental approaches to the problems which are being investigated further. Among these are: (*1*) Will a challenge with a large inoculum in one cornea in an animal rendered immune by an intradermal injection reduce the immunity to a smaller challenge of the second cornea, given at the same time, or subsequent to, the large challenge in the first cornea; (*2*) will the addition of a large number of dead bacilli to a small live inoculum affect the multiplication of this inoculum in the cornea of an immune animal; and (*3*) will there be multiplication of a small inoculum in the cornea of a mouse which has been injected intravenously a few days previously with a dose which will ultimately kill the animal?

Summary

The mouse corneal method was used to study quantitatively the development of immunity to tuberculosis. The immunity resulting from a previous vaccination was assessed by the development of macroscopic lesions and the extent of bacillary multiplication in the challenged eye.

It was found that some degree of immunity could be demonstrated with a challenge given forty-eight hours after vaccination and that this gradually increased to a maximum about a month after vaccination. At this stage a small inoculum (about 500 organisms) underwent no multiplication whatever in the challenged eye. Immunity sufficient to prevent the development of any macroscopic lesions in challenged eyes was observed up to thirteen months after vaccination, the longest period studied.

The introduction of a larger challenge dose in immunized animals was followed by bacterial multiplication and the production of a macroscopic lesion, i.e., the larger challenge did overcome the immunity, although the extent of the multiplication lagged behind that observed with a similar dose of bacilli in non-immune animals. Detailed microscopic investigation showed that, when a large inoculum overcame immunity, this was not due to insufficient phagocytic invasion and that under these conditions intracellular multiplication of organisms, with ensuing destruction of the cells, did occur.

The production of immunity with bacilli killed by various methods was investigated. The best results were obtained with phenol-killed organisms which, in large doses, produced an immunity almost comparable with that seen with live organisms.

Sumario

La Inmunidad en la Tuberculosis

Se empleó la técnica de la córnea murina para estudiar la formación de inmunidad a la tuberculosis. La inmunidad derivada de una vacunación anterior fué justipreciada por medio de la aparición de lesiones macroscópicas y la extensión de la proliferación bacilar en el ojo provocado.

Observóse que cabía descubrir alguna inmunidad con una provocación aplicada cuarenta y ocho horas después de la vacunación y que la misma alcanzaba gradualmente su máximo

aproximadamente al mes de la vacunación. En esta etapa un inóculo pequeño (unos 500 microbios) no experimentó la menor proliferación en el ojo provocado. Hasta a los trece meses de la vacunación, el período más largo observado, se observó en los ojos provocados una inmunidad suficiente para impedir la aparición de toda lesión macroscópica.

La introducción de una dosis provocadora mayor en los animales inmunizados fué seguida de multiplicación bacteriana y de la producción de una lesión macroscópica, es decir, que una provocación mayor agobió la inmunidad, aunque la magnitud de la proliferación fué inferior a la observada con una dosis semejante de bacilos en animales sin inmunidad. Una detallada investigación microscópica reveló que, cuando un inóculo grande sobrepujaba la inmunidad, esto no se debía a insuficiente invasión celular y que, en tales circunstancias, sí había proliferación intracelular de los gérmenes, con la consiguiente destrucción de las células.

Se investigó la producción de inmunidad con bacilos matados con varias técnicas. Los mejores resultados fueron los obtenidos con microbios matados con fenol, que, a dosis grandes, produjeron una inmunidad casi idéntica a la observada con microbios vivos.

Resume

Immunité dans la tuberculose

L'épreuve de la cornée chez la souris a été utilisée pour étudier quantitativement le développement de l'immunité à la tuberculose. L'immunité provoquée par une vaccination antérieure était évaluée selon le développement des lésions macroscopiques et l'étendue de la multiplication des bacilles dans l'oeil testé.

On a découvert qu'un certain degré d'immunité pouvait être démontré par le test pratiqué au niveau de la cornée 48 heures après la vaccination; on a constaté que l'immunité augmentait graduellement jusqu'à un maximum d'un mois environ après la vaccination. A ce stade, un petit inoculum (environ 500 bacilles) ne subissait aucune multiplication quelle qu'elle soit dans l'oeil testé. Une immunité suffisant à prévenir le développement de toute lésion macroscopique dans les yeux testés a été observée jusqu'à treize mois après la vaccination, période la plus longue qui ait été étudiée.

L'introduction d'une dose d'épreuve plus importante chez les animaux immunisés était suivie d'une multiplication bactérienne et la production d'une lésion macroscopique, c'est à dire que la dose d'essai plus élevée annulait l'immunité. Toutefois, la valeur de la multiplication restait inférieure à celle qui était observée avec une dose similaire de bacilles chez les animaux non immunisés. Des recherches microscopiques détaillées montraient que lorsqu'un inoculum important annulait l'immunité, ce fait n'était pas dû à l'insuffisance de l'invasion de la cellule et que dans ces conditions, il se produisait une multiplication intracellulaire des bacilles avec destruction subséquente des cellules.

Les auteurs ont étudié la production de l'immunité avec des bacilles tués par diverses méthodes. Les meilleurs résultats ont été obtenus avec les bacilles tués par le phénol; à dose élevée, ils produisaient une immunité presque semblable à celle que l'on obtenait avec les organismes vivants.

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RELATIONSHIP OF ALLERGY TO GROSS LUNG DISEASE AND CULTURABLE BACILLI IN TUBERCULOUS MICE[1, 2]

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INTRODUCTION

It has been reported previously (1) that, compared with guinea pigs, C57 black mice are resistant to tubercle bacilli lodging in organs other than the lung, but that every viable bacillus reaching this highly susceptible organ is probably capable of initiating a progressive tubercle (2). Therefore the lung has been selected as the basis of a detailed study of the progress of murine tuberculosis.

The discovery that 0.03 ml. of Old Tuberculin (OT) diluted 1:25 in saline injected into the footpad was a satisfactory test for tuberculous allergy in mice led to a study of the chronologic relationship of allergy to survival (3). Deaths of animals infected with less than 2,000 viable bacilli were confined either to the preallergic or to the terminal anergic phases of the disease, and no animal died while showing a positive footpad reaction. Moreover, the progress of lung lesions appeared to follow closely the observed mortality pattern.[3]

The present experiments were designed to determine whether a correlation could be demonstrated between the preallergic, allergic, and terminal anergic phases of the disease and the degree of activity of the tuberculous processes in the lungs.

Data relating the ratio of viable to stainable bacilli in the lungs of these animals have been published separately (4).

MATERIALS AND METHODS

All of the manipulations involving living tubercle bacilli were carried out under a bacteriologic hood. The mice, which belonged to the C57 black strain used in earlier studies, were infected intranasally with small to moderate doses of dispersed cultures of H37Rv. Anesthetized mice received three drops (0.06 ml.) from a dropping pipette delivering 50 drops per ml. (1–3).

Footpad tests consisted of injecting approximately 0.03 ml. of OT[4] diluted 1:25 in saline into a footpad of alternate hind feet at weekly intervals during the period of observation (3). Reactions in the form of a hyperemic swelling of the foot were recorded forty-eight hours after inoculating the tuberculin. The control tests were performed on uninfected mice which failed to react to repeated injections of 1:25 OT.

Gross lung changes were assessed as the increase in the combined weight of lung lobes and

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[3] When very much larger numbers of bacilli, e.g., 0.5 ml. of a ten-day dispersed culture, diluted 10^{-1}, were injected intravenously (a common method of infecting mice), a small proportion of animals reaching the allergic stage actually died before becoming anergic. The great majority, however, followed the above pattern (to be published).

[4] Commonwealth Serum Laboratories, Melbourne. The Old Tuberculin was prepared from cultures grown on synthetic medium and dispensed for bovine herd-testing.

hilar lymph nodes over the average lung weight of normal adult C57 mice (0.27 gm.). This afforded an accurate means of recording the visible changes in the organ. The entire bronchial tree, minus the heart, was rinsed in saline, rested a few seconds on sterile filter paper to absorb surplus fluid, and transferred to a weighed, sterile Petri dish.

Viable counts were made on the lobes of the lungs, excluding the lymph nodes. Lobes from individual or groups of mice were homogenized for two minutes in a screw-topped Waring Blendor with 10 ml. of 0.1 per cent albumin water per lung, giving an initial dilution of 10^{-1} per lung. One minute after stopping the machine, a sample was removed for dilution and plating on Löwenstein's medium (1).

The term "flare" is used in the present paper to indicate rapid multiplication of tubercle bacilli occurring within progressive lung lesions.

Results

The results presented here were obtained with C57 mice infected intranasally with either moderate or small doses of tubercle bacilli. In all other respects the animals received similar treatment. Of 52 mice receiving the larger dose (12,500 viable units), about one-third showed severe early involvement and either died or were sacrificed while moribund. The rest remained well and were sacrificed at selected intervals after developing a positive footpad reaction.

Most of the 65 animals which received the smaller infecting dose (500 viable units) survived the preallergic phase. The progress of the disease was subsequently assessed at intervals through the allergic phase into the terminal anergic phase of the disease.

The results appear in tables 1 and 2 and, inasmuch as they illustrate collectively the infection pattern of C57 mice infected under these conditions, they may be considered together.

The objective was to study the rate of development of pulmonary lesions and of the bacilli contained in them in relation to the time that elapsed since infection and the onset and disappearance of footpad sensitivity. Accordingly, groups of mice were killed and examined at predetermined intervals. Animals were also examined as they died and, when necessary, additional healthy animals were sacrificed for comparison with these.

Because it was desired to ascertain the average response of large numbers of mice to infection under these conditions, and in order to limit the technical problems arising from numerous individual examinations undertaken at any one time, the lungs were examined in groups. Thus, the data presented are those obtained from mean lung weights and counts of bacilli from pooled lung tissue, except when the contrary is indicated in the tables.

For convenience of presentation and discussion, the mice have been arranged in three categories, viz., "pre-allergic," including mice that died or were killed before a positive footpad reaction was demonstrated; "allergic," including those showing a positive reaction at the time of sacrifice; and "terminal anergic," those previously showing a footpad reaction, but failing to do so on at least one occasion before death or sacrifice.

Preallergic phase: The preallergic period lasts from four to eight weeks, depending largely on the size of the infecting dose. In the present series it was characterized by a rapid increase in lung weight and in viable count over the

TABLE 1

RELATIONSHIP OF FOOTPAD SENSITIVITY TO GROSS LUNG DISEASE AND VIABLE TUBERCLE BACILLI IN C57 MICE INFECTED INTRANASALLY WITH 12,500 BACILLI (Log. 4.09)

Group	Number of Mice	Weeks Since Infection	Footpad Reaction to 1:25 OT in Weeks Preceding Death					Physical State of Mice	Fate	Extent of Disease in Lungs				Category
			4	3	2	1	At Death			Average Weight of Lungs and Nodes (Normal Average 0.27 gm.)		Log. Viable Count (Lobes)		
										gm.				
A	5	1	·	·	·	−	−	Well	Killed	·		5.06		Pre-allergic
B	5	2	·	·	−	−	−	Well	Killed	0.38		7.20		
C	2	2½	·	·	−	−	−	Sick	Killed	0.55		9.12		
D	5	3	·	−	−	−	−	Well	Killed	0.41		7.38		
E	5	3	·	−	−	−	−	Sick	Killed	0.69		9.28		
F	5	3	·	−	−	−	−	Sick	Killed	0.69		9.53		
G	2	3½	·	−	−	−	−	Sick	Died	0.97		9.39		
H	1	4	−	−	−	−	−	Sick	Died	0.95		9.42		
I	1	4½	−	−	−	−	−	Sick	Died	0.75		8.92		
J	3	4	−	−	−	−	+	Well	Killed	0.63	Mean 0.60 gm.	7.91	Mean 6.87 gm.	Allergic
K	4	4	−	−	−	+	+	Well	Killed	0.56		7.09		
L	3	5	−	−	+	+	+	Well	Killed	0.72		6.91		
M	5	5	−	−	+	+	+	Well	Killed	0.60		6.59		
N	3	6	−	−	+	+	+	Well	Killed	0.53		6.70		
O	3	7	−	+	+	+	+	Well	Killed	0.60		6.50		

period, best seen in groups A to I, which received the heavier dose (table 1). Sixteen dead or moribund mice examined two and one-half to four and one-half weeks after infection showed an average lung weight of 0.73 gm., which is three times the normal weight, and an average count of 2.2×10^9 viable tubercle bacilli, about 200,000 times the infecting dose.

These changes, constituting the "preallergic flare," culminate either in death or in arrest of the disease.

Allergic phase: Whether they received the small or the larger infecting dose, animals surviving to the stage at which a positive footpad reaction appeared showed approximately the same degree of infection at the time of conversion (*see* allergic group, tables 1 and 2). This was a serious but less severe degree of lung consolidation than was encountered in the preallergic mice, suggesting that the onset of "immunity" probably preceded by an undetermined interval the development of a detectable footpad reaction.

Fifty-eight mice were sacrificed (21, table 1; and 38, table 2) from one to forty-nine weeks after their first footpad reaction, all having shown a positive reaction each week until they were killed. The average lung weights of all of these mice were remarkably consistent, showing no significant increase beyond that reached at the time of conversion, and the bacillary population seldom equalled that of mice sacrificed at the time of conversion. The mean lung weights of the larger and

TABLE 2

RELATIONSHIP OF FOOTPAD SENSITIVITY TO GROSS LUNG DISEASE AND VIABLE TUBERCLE BACILLI IN C57 MICE INFECTED INTRANASALLY WITH 500 BACILLI (Log. 2.70)

Group	Number of Mice	Weeks Since Infection	Footpad reaction to 1:25 OT in Weeks Preceding Death			Physical State of Mice	Fate	Extent of Disease in Lungs				Category
			2	1	At Death			Average Weight of Lungs and Nodes (Normal Average 0.27 gm.)		Log. Viable Count (Lobes)		
								gm.				
A	5	2	−	−	−	Well	Killed	0.36		7.10		Preallergic
B	5	4	−	−	−	Well	Killed	0.61		7.54		
C	2	5	−	−	−	Sick	Killed	0.90		9.23		
D	2	6	−	−	−	Sick	Killed	.		9.20		
E	5	4	·	·	+	Well	Killed	0.55		7.53		Allergic
F	3	5	+	+	+	Well	Killed	0.52		6.83		
G	3	6	+	+	+	Well	Killed	0.53		6.61		
H	5	10	+	+	+	Well	Killed	0.53		5.58		
I	5	13	+	+	+	Well	Killed	0.55	Mean 0.55 gm.	6.11	Mean 6.40	
J	10	15	+	+	+	Well	Killed	0.56		6.14		
K	2	16	+	+	+	Well	Killed	0.59		6.25		
L	3	24	+	+	+	Well	Killed	.		6.74		
M	1	49	+	+	+	Well	Killed	0.50		6.32		
N	1	49	+	+	+	Well	Killed	0.55		6.34		
O	1	13	+	−	·	Sick	Died	0.60		6.40		Terminal Anergic
P	1	13	−	−	−	Sick	Killed	1.0		9.11		
Q	1	13	−	−	·	Sick	Died	0.90		9.12		
R	1	15	−	−	·	Sick	Died	0.95		9.70		
S	1	15	+	−	−	Well	Killed	0.68	Mean 0.84	7.18	Mean 8.19	
T	2	15	−	−	−	Sick	Killed	0.60		7.59		
U	1	16	−	−	−	Sick	Killed	0.75		7.88		
V	1	16	−	−	·	Sick	Died	1.1		9.48		
W	2	17	−	−	−	Sick	Killed	0.82		7.60		
X	1	24	−	−	−	Sick	Killed	0.92		8.02		
Y	1	29	−	−	·	Sick	Died	1.25		9.07		

small infecting-dose groups, respectively, were 0.6 gm. (21 mice) and 0.55 gm. (38 mice). The mean viable counts for these groups were log. 6.87 and log. 6.40, respectively.

As these figures were based on the time that elapsed between infection and death and failed to allow for the observed variation from mouse to mouse in the time of conversion to the positive footpad state (3), an additional 52 mice were examined during the allergic stage. Originally infected with approximately 10,000 viable units, these animals were sacrificed at intervals based on the time of the first positive footpad reaction. The average lung weights and viable counts obtained from this group up to eight weeks after conversion are set forth in table 3. This result strengthens the impression gained above that the activity of the

TABLE 3

STATUS OF LUNG DISEASE DURING THE ALLERGIC PHASE IN C57 MICE INFECTED INTRANASALLY WITH 10,000 BACILLI (Log. 4.00)

	Duration of Positive Footpad Reaction (weeks)							
	1	2	3	4	5	6	7	8
Mean lung weight (gm.)	0.60	0.68	0.70	0.59	0.67	0.70	0.68	0.73
Log. mean viable count	6.17	6.06	6.14	5.44	5.50	6.43	5.86	6.12
Number of mice	6	7	5	10	5	6	11	2

lung lesions was virtually arrested at the approximate level reached at the time of conversion and remained in this state for many weeks.

This stage represents the period of immunity or "steady state."

Terminal anergic phase (table 2): In earlier experiments (3) it was reported that mice infected intranasally with doses of 2,000 tubercle bacilli or less might die either before the development of measurable allergy or shortly after it had waned, but not while it was present.

It was also stated that the mice invariably died within one to four weeks of losing the ability to give a positive footpad reaction. At that time, the bacillary content of the lung lesions was assessed in most cases by direct observation of sections stained by fluorescence microscopy. The conclusion then reached regarding bacillary numbers was that death was not usually preceded by massive multiplication of bacilli or by gross changes in the degree of lung consolidation compared with footpad-positive survivors at the same stage of the disease.

Subsequent observations, however, in which viable counts were regularly employed, indicated that a terminal increase in lesions and bacilli could be demonstrated and was, in fact, the usual sequel to anergy, especially when death failed to follow rapidly after loss of footpad sensitivity.

The present results are interpreted as providing evidence that the "steady state" of the lesion observed in the allergic phase gives way to a fresh period of progressive development when anergy supervenes, but that this progress becomes obvious only if the terminal anergic stage persists for several weeks prior to death. The onset of the terminal change may be overlooked if this stage is short, as it was in the majority of mice in the experiments reported previously.

Thus, of 13 animals which died in the anergic state between the thirteenth and twenty-ninth weeks of the disease (table 2), only one (a rapid death) presented a lung weight-viable count ratio of the type found in the allergic phase. The rest showed a much greater lung involvement and up to a thousand times the number of bacilli detected at any time during the allergic phase (mean lung weight = 0.84 gm.; mean viable count = log. 8.19, compared with 0.55 gm. and log. 6.40, respectively, during this allergic phase).

Because post-mortem contamination of the lungs frequently resulted in failure to obtain a satisfactory viable count when animals were not examined immedi-

ately after death, some mice were killed when it appeared unlikely that they would survive overnight. The counts in these cases tended to be lower than in those allowed to die naturally. Nevertheless, the average lung weights and viable counts of these mice were so much greater than those of any groups that were killed in the allergic stage, even up to forty-nine weeks after infection, that it seems justifiable to conclude that a terminal recrudescence of the disease is the usual accompaniment of terminal anergy in this strain of mice under these experimental conditions.

The degree of variation from the mean lung weights and viable counts obtained by these group studies is yet to be determined, but the trends that emerge appear to the present writers to be indicative of the usual course of the disease under the experimental conditions employed.

This stage is referred to as the "terminal anergic flare."

Discussion

Except when extremely heavy infecting doses have been given, tuberculous mice may be separated, on the basis of time of death, into an early and a late group. The proportion of early to late deaths and the duration of the interval between, in which few or no deaths occur, are governed by many factors, particularly the mouse strain and the size of dose, route of inoculation, and virulence of the infecting organism.

This phenomenon, resembling a double epidemic, which is familiar to workers in experimental mouse tuberculosis, is most obvious in C57 mice when they have been infected with about 2×10^4 organisms intranasally or 2×10^6 organisms by the intravenous route. The peak of early deaths occurs about the third week; and the death rate then falls, usually to zero, by the sixth week. At this stage surviving mice appear physically well and may remain so for weeks or months and occasionally up to two years.

Subsequent regular inspection will reveal various numbers of sick mice from time to time, which are then observed to deteriorate quickly and to die, usually within a few weeks of the onset of clinical illness. All of the mice will eventually die in this manner, although the time at which reversion to the anergic state will occur in individual mice cannot be anticipated. By increasing or decreasing the infecting dose, the bulk or even all of the deaths can be induced to occur either in the early or the late stage.

Footpad tests performed at weekly intervals show that these events are closely linked with tuberculin sensitivity in the following respects:

All of the early deaths occur in mice that fail to develop a footpad reaction. The normal preallergic flare in these mice remains unchecked by the mobilization of the immunity mechanisms, so that lung consolidation and bacillary multiplication continue until virtually the entire effective lung tissue is involved. The lower preallergic death rate observed when small infecting doses are used is believed to be determined by the reduced lung involvement. In other words, if a mouse lives long enough, it will enter the immune phase.

The appearance of a footpad reaction indicates that the animal will survive the

preallergic period and enter a phase of relative immunity of unpredictable duration. This may last as short a time as a few weeks or as long as two years. It is characterized by a "steady state" in the lung lesions in contrast to the preallergic flare and by the persistence of the footpad reaction.

It is observed that the state of the disease in animals sacrificed when footpad sensitivity first appears is less advanced than that in preallergic sick or dead mice infected for the same period. This suggests that the inhibitory action of the immune defenses has probably been operative for at least a short time before tuberculin sensitivity is manifested.

The termination of the steady state in individual mice and the onset of a terminal resurgence of the disease are believed to be indicated by a brief diminution, then disappearance, of the footpad reaction. At the present level of dosage no animal died while footpad positive, but death followed within a few weeks of the disappearance of the reaction. At the moment no explanation is offered for this rather abrupt transition to the anergic state, but the evidence suggests that the immune state also breaks down at about the same time.

In the present studies, a proportion of the anergic mice were found to have a superinfection, usually with *Pasteurella pneumotropica*, in addition to the terminal flare of tubercle bacilli. Because it was not possible to determine the extent to which this complication contributed to the final lung weight or to the death of the animal, all of these animals have been excluded from the present results. This accounts in part for the relatively small number of results in this section of the experiment.

At the present time an experiment is in progress in which half the animals are maintained on continuous chloramphenicol therapy in an attempt to assess the contribution of nontuberculous superinfection, if any, to the character of the terminal stages of the tuberculous disease.

The present evidence is interpreted as showing that the steady state present during the allergic phase is replaced by renewed multiplication of bacilli and rapid extension of the lesions. To what extent the duration of the terminal stage depends on the amount of functional lung tissue remaining at the end of the allergic phase is yet to be determined, but it is expected that it may be a contributing factor. If this should prove to be the case, then death from tuberculosis in the mouse, whether in the preallergic or the terminal anergic stage, could well be "physiologic" rather than "toxic" in origin.

Aside from this speculation, the longer the animal survives the loss of footpad sensitivity, the greater the increase in lung weight and in bacillary numbers. It is hoped that current experiments will indicate whether the rate of progress is comparable with that observed in the preallergic phase.

It is not suggested that these results make any real contribution to the solution of the general problem of the importance of allergy in the immune response to tuberculosis. It seems, however, that the mouse is a particularly suitable animal for studying the relationship of these two phenomena. Allergic damage as an accompaniment of infection is relatively slight in the mouse, and there is little tendency, especially after intranasal inoculation, for vital organs other than the lung to be involved to an extent that might lead to premature death.

Especially if small infecting doses are used, so that animals enter the allergic phase with an adequate amount of undamaged lung tissue, it is to be expected that death, when it occurs, will be the result of the "normal progress" of the disease as opposed to what might be termined a "premature death" of the type commonly

encountered in tuberculous guinea pigs, arising from some accident to a vital organ, e.g., rupture of the spleen or liver.

In view of this, it is suggested that loss of allergy prior to death, and at about the same time as the immune response begins to fail, may be the true pattern of the uncomplicated disease. Attempts to provide evidence in support of this hypothesis will be published separately (5).

Preliminary studies have been made of the allergic response of mice of the C57 black, Rockefeller Swiss, and Rockefeller Institute strains to large doses of tubercle bacilli intravenously. These suggest that the method used in the present experiment, viz., a small intranasal infecting dose, was probably important in enabling the apparent regularity of the terminal anergic flare to be demonstrated.

Recognition of the fact that a challenging dose of virulent bacilli, when given to immunized and control groups, will, per se, give rise to an immune phase, poses a problem of interpretation that has led to some confusion.

It is obvious that comparison of death rates in immunized and control groups cannot be valid beyond the end of the preallergic phase of the challenge infection. The use of the footpad test on the control group would indicate when this stage has been reached.

Whether the solution to this difficulty lies in giving large enough challenge doses intravenously to ensure that all of the controls die in the preallergic stage, or in giving small challenge doses intranasally and assessing the result in terms of lung involvement three or four weeks later, is yet to be determined.

In conclusion it is pointed out that the above results are based for the most part on examination of groups of mice rather than of individuals. It is not known, therefore, to what extent intra-group variations occur on either side of the mean result presented. Further experiments are being undertaken in this connection.

Summary

The correlation previously reported between the allergic phase of mouse tuberculosis and the ability of mice to survive intranasal infection with small numbers of tubercle bacilli has been confirmed. Progress of gross lung involvement, which paralleled bacillary multiplication, was arrested with the appearance of footpad sensitivity. Mice sacrificed during the allergic phase, from four to forty-nine weeks after infection, showed arrested development of lung lesions regardless of the duration of the allergic phase.

The stage of terminal anergy was characterized by further progress of gross lung lesions and a proportional increase in bacillary numbers up to one thousand times the average numbers recorded in mice sacrificed during the allergic phase. Demonstration of this phase was facilitated by using a small infecting dose intranasally.

Sumario

Relación de la Alergia con la Enfermedad Pulmonar Macroscópica y los Bacilos Tuberculosos Viables en los Ratones Tuberculosos

Se ha confirmado la correlación señalada anteriormente entre la fase alérgica de la tuberculosis murina y la capacidad de los ratones para sobrevivir la infección intranasal

con pequeñas cantidades de bacilos tuberculosos. Con la aparición de la sensibilidad plantar se estacionó la evolución de la invasión pulmonar macroscópica, que emparejaba la proliferación bacilar. Los ratones sacrificados durante la fase alérgica, de cuatro a cuarenta y nueve semanas después de la infección, revelaron estacionamiento del desarrollo de las lesiones pulmonares, independientemente de la duración de la fase alérgica.

El período de anergia terminal se caracterizó por nuevo avance de las lesiones pulmonares macroscópicas y aumento proporcional de los números de bacilos hasta mil veces más de las cifras medias registradas en los ratones sacrificados durante la fase alérgica. Se facilitó el descubrimiento de esta fase usando intranasalmente una pequeña dosis infectante.

Resume

Les rapports entre l'allergie, les lésions pulmonaires macroscopiques et les bacilles viables chez les souris tuberculisées

Confirmation de la corrélation antérieurement exposée entre la phase allergique de la tuberculose de la souris et l'aptitude des souris à survivre après l'inoculation nasale d'un petit nombre de bacilles. Les progrès des lésions macroscopiques pulmonaires, comparables à la multiplication bacillaire s'arrêtaient au moment de l'apparition de la réaction allergique au niveau de la patte. Les souris sacrifiées durant la phase allergique, de quatre à quarante-neuf semaines après l'inoculation, présentaient des lésions pulmonaires stabilisées, quelle que soit la durée de la phase allergique.

Le stade d'allergie terminale était caractérisé par une reprise du développement des lésions macroscopiques pulmonaires avec augmentation proportionnelle de la population bacillaire, qui atteignait jusqu'à mille fois les chiffres moyens enregistrés chez les souris sacrifiées durant la phase allergique. La démonstration de cette phase était facilitée par l'inoculation intra-nasale d'une petite dose de bacilles.

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IMMUNITY, NATURAL ANERGY, AND ARTIFICIAL DESENSITIZATION IN EXPERIMENTAL TUBERCULOSIS[1, 2]

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Introduction

Experimental tuberculosis in mice and guinea pigs, being almost invariably fatal, differs from the naturally occurring disease with its low death rate in man. Nevertheless, it is important to recognize the existence of a phase of the experimental disease in which survival is prolonged by the development of specific immunity. This phase is characterized by a slowing down or even a temporary arrest of the disease process, so that the animal remains apparently well for its duration. The end of the immune phase is marked by rapid physical deterioration and death within a relatively short period. The commonly used index of the onset of immunity to tuberculosis, in experimental animals as well as in natural infection or vaccination in man, is the appearance of allergy to tuberculin. There is less certainty, however, whether allergy and immunity disappear at about the same time in the terminal stages of the disease.

The knowledge that tuberculin allergy may also be associated with severe and extensive tissue damage, particularly in man and guinea pigs, has led to a preoccupation with the significance of allergy per se in the immune response. From this arose a tendency to regard immunity against tuberculosis as differing fundamentally from that against other diseases in not being mediated by specific antibodies (in addition to cellular—including allergic—and nonspecific humoral phenomena). This view has many adherents, despite the fact that the evidence on which it is based is not completely satisfying.

As the basis for a reconsideration of existing theories, it is suggested that allergy may be nothing more than one aspect of the immune response associated with specific changes in the activity of phagocytic cells. Moreover, it seems possible that allergy is not a simple phenomenon, but rather a complex of reactions, some of which are detrimental to certain hosts under certain conditions. If this were so, it would have an important bearing on the question occupying the attention of many workers in this field regarding the possibility of producing immunity in the absence of the harmful aspects of the allergic response.

Studies with C57 mice infected intranasally with less than 1,500 virulent bacilli of strain H37Rv (1) suggested the existence of a close relationship between the duration of the immune phase and of the phase of footpad sensitivity

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in this species. Under these conditions, all deaths were found to occur either before allergy appeared or after it had disappeared, i.e., in the "preallergic" or in the "terminal anergic" phases of the disease. No animal died while showing a positive footpad reaction to 0.03 ml. of 1:25 Old Tuberculin (OT). The common concept that terminal anergy is nothing more than a reduced ability on the part of a moribund individual to react to allergic stimuli was not supported by the above findings. On the contrary, it was found that the rapid physical deterioration characteristic of the terminal stages of the disease usually followed rather than preceded the loss of footpad sensitivity to OT.

When the progress of gross lung lesions and the rate of bacillary multiplication within them were studied in relation to the onset and waning of footpad sensitivity, a positive correlation was found between the clinical picture and the pathologic and bacteriologic findings in the lungs (2).

In the preallergic phase, a proportion of the mice, depending on the size of the infecting dose, were unable to cope with what the present writer regards as the preallergic flare. Death during this stage was found to be associated usually with extensively consolidated lungs teeming with tubercle bacilli, so that a factor contributing largely to death was inadequacy of functional lung tissue. All animals that survived the preallergic stage developed footpad sensitivity at or after the third or fourth week of the disease. At this time the amount of lung tissue involved and the bacillary population, although often somewhat higher than at any subsequent stage of the allergic phase, were both lower than in tuberculin-negative animals, sick or dead at the same stage of infection. This apparent arrest of the disease was evident as long as allergy was demonstrable, i.e., up to forty-nine weeks in the experiments under discussion.

Footpad sensitivity was replaced by a state of anergy to 1:25 OT and progressive clinical illness in all mice that had not been previously sacrificed. When allowed to die, or when sacrificed in a moribund condition, these animals showed a recrudescence of lung lesions, accompanied by renewed multiplication of bacilli, so that viable counts were up to one thousand times the average counts obtained from allergic mice.

These results suggested the possible existence of a similar basic disease pattern for uncomplicated tuberculosis in man and in guinea pigs, and a survey of the literature provided some evidence that appeared to warrant further observations on the behavior of these two species (3, 4). As a preliminary step, the lungs of guinea pigs infected intranasally with a small number of tubercle bacilli were studied to determine whether a similar correlation could be shown to exist in this species between the rate of multiplication of bacilli and the duration of the allergic phase. The present paper records the results of this study and of subsequent experiments comparing the influence of naturally occurring and artificially induced anergy on the course of tuberculosis in guinea pigs and in mice, re-examining and extending the results of Woodruff and his colleagues (3–6) and of Follis (7). It is emphasized that, although gross changes in the spleens and livers of guinea pigs were recorded, the observations on the rate of multiplication of bacilli were confined to the lungs in these animals and in mice.

Materials and Methods

The previous description of most of the materials and methods, including the origin and maintenance of the C57 black mice and the guinea pigs, and of the strain of *Mycobacterium tuberculosis* H37Rv, should be referred to for precise details omitted here (1, 2, 8). Both mice and guinea pigs were infected intranasally under ether-chloroform anesthesia with eight- to ten-day dispersed cultures standardized by a colorimetric method (1, 8) and diluted to give an anticipated number of viable bacilli. The actual infecting dose was checked by viable counts (8) and was usually well within the anticipated range. Mice received a total volume of 0.06 ml. (3 drops from a dropping pipette delivering 50 drops per ml.), and guinea pigs were given twice that volume because of the greater area of lung parenchyma to be reached by the suspended bacilli.

Tuberculin tests were carried out with Old Tuberculin[4] produced on synthetic medium and dispensed for use in bovine herd-testing. Guinea pigs were tested at weekly intervals on the shaven dorsal skin with OT diluted 1:50 in saline, while footpad tests (1) were carried out each week on alternate hind feet of the mice using 1:25 OT. The results in both species were recorded as positive (+), intermediate (I), or negative (−), while +N in guinea pigs indicates the additional occurrence of necrosis. The intermediate designation, which is noted frequently in mice and occasionally in guinea pigs during the onset and waning of allergy (1), was applied to a reaction which failed to show sufficient induration and erythema to be recorded as positive.

Gross organ changes: The development of lesions in the lungs was assessed in terms of progressive weight increments over a predetermined norm for uninfected animals of comparable age (2). This afforded an effective means of recording the visible changes in the organ. Lobe weights and lymph node weights (*see* tables) were determined by rinsing the entire bronchial tree minus the heart in sterile saline, resting a few seconds on sterile filter paper, then snipping off the lobes into a weighed, sterile Petri dish. The residue, consisting of the hilar nodes, the lower trachea and bronchi, a portion of the esophagus, and (in young animals,) the thymus gland, was weighed and recorded as "nodes." In cases in which the entire lung weight is recorded, "nodes" and lobes were not separated.

Gross changes in the livers and spleens of guinea pigs were recorded on a visual basis at autopsy, the scores 1, 2, 3, or 4 corresponding respectively to slight, moderate, severe, or extreme macroscopic involvement of the organ.

Viable counts: After weighing, the lobes, either from one animal or (as noted in the text) from several animals, were suspended in 10 ml. of 0.1 per cent albumin water per lung and homogenized for two minutes in a screw-topped Waring Blendor. One minute after stopping the machine, a sample was removed for dilution and plating (8). To avoid loss of data owing to the occasional presence of contaminants in dead animals, another count was made after treating the homogenate with a final concentration of 2 per cent sodium hydroxide for thirty minutes and neutralizing. When the numbers could not be determined directly on the untreated tissue suspension, the number of viable bacilli was estimated from the second count on the basis that sodium hydroxide destroys about 80 per cent of the original number of tubercle bacilli under these conditions (9). These occasions were rare and are noted in the appropriate section.

Tuberculin treatment: In selecting the daily dose of OT to be used in the guinea pigs, consideration was given to a routine of injections that would not give rise to undue discomfort and damage (as usually results from large subcutaneous injections of OT daily) but would suppress the development of skin sensitivity to 1:10 OT intradermally. Treatment with tuberculin commenced at the same time as infection, with an initial dose of 0.2 ml. rising to 1 ml. daily after one week. From the eighth week, the dose was reduced to 1 ml. of OT every three days because it then became apparent that the effects of prolonged treatment were maintained long after injections were discontinued (table 2). In view of present experience, it may well be that smaller and less frequent injections may achieve the same result.

[4] Commonwealth Serum Laboratories, Melbourne, Australia.

A suitable parallel treatment of the control animals was considered carefully. Follis (7) gave an equal volume of glycerin but this seemed unsuitable because, despite the theoretical content of 50 per cent glycerin in OT, it is known that a large part of the original glycerin in the broth is metabolized by the growing organisms. It was finally decided to inject 0.3 per cent phenol saline, the OT having been preserved with 0.5 per cent phenol, which is slowly destroyed in contact with tuberculoproteins. This procedure compensated in part for the trauma of daily injection without introducing an enormous amount of glycerin certainly not present in OT, which in the experiments of Follis appeared capable of influencing the course of the disease per se.

In mice the onset of tuberculin treatment was delayed for two weeks after infection, as experience had shown that allergy was not detectable before that time and indeed was seldom apparent before the fourth week. The daily dose selected for this species was 0.2 ml., with phenol saline as a treatment for the controls.

Results

Relationship of Allergy to Gross Disease and to Culturable Bacilli in the Lungs of Guinea Pigs Infected Intranasally

Guinea pigs infected intranasally with approximately 5,000 bacilli were skin-tested at weekly intervals with 1:50 OT until death occurred fourteen to twenty-three weeks later. There were, of course, no preallergic deaths. In table 1, these animals have been grouped according to skin sensitivity in the final stage of the disease. A comparison was made of gross lesions in the lungs, spleens, and livers and of the bacillary population of the lung in these two groups.

Although the number of animals involved was small, the results were in accord with the observations of Woodruff and Willis (5, 6) and are therefore considered to provide significant evidence. Six of 11 animals in the group died while still sensitive to 1:50 OT, but it was clear that a fatal complication (total lung involvement or rupture of spleen or liver) had ensued in at least 5 of these animals. The sixth showed only moderate pleural, pericardial, and abdominal effusions of doubtful significance in relation to a possible cause of premature death. All were in fair to good physical condition at the time of death, with a relatively low bacillary population in the lungs. The remaining 5 developed natural anergy to 1:50 OT two to four weeks before death and showed the progressive emaciation and renewed multiplication of bacilli characteristic of the terminal anergic mouse deaths (1, 2).

Unlike the "steady state" observed in allergic mice (2), the gross involvement of lungs, spleens, and livers apparently progressed during the allergic period, despite the arrest of bacillary multiplication observed in the lungs (*see also* table 2, hypersensitive group). This observation raises the question whether allergic inflammation, which is relatively unimportant in mice (1), actually determines the character of the gross disease in guinea pigs during the allergic phase.

It is clear that this group of guinea pigs failed to show the regular correlation between the presence of allergy and the ability to survive that was observed in mice. Nevertheless, if allowance is made for the greater tendency in guinea pigs for excessive organ damage to give rise to prematurely fatal complications, the sequence of events would not be inconsistent with the basic mouse pattern.

TABLE 1

NATURAL DEATHS IN ALLERGIC AND ANERGIC GUINEA PIGS INFECTED INTRANASALLY WITH 5,000 BACILLI (H37Rv)

Guinea Pig Number	Duration of Disease	Tuberculin Test in Preceding Weeks						Physical Condition	Lung Examination		Gross Involvement		Remarks
		6	5	4	3	2	1		Gross Lesions	Viable Counts* $\times 10^6$	Spleen	Liver	
	weeks												
71b	17	+	+	+	+	+	+	Good	2	10.5	4	1	Ruptured spleen
52b	18	+	+	+	+	+	+	Fair	4	35.8	2	2	Total lung involvement
83b	19	+	+	+	+	+	+	Good	4	13.2†	4	3	Total lung involvement
71a	21	+	+	+	+	+	+	Good	3	9.7	3	2	Effusions in pericardial, pleural, and abdominal cavities
82d	22	+	+	+	+	+	+	Fair	2	160	3	4	Ruptured liver
71c	23	+	+	+	+	+	+	Fair	4	55	3	2	Total lung involvement
83a	14	+	+	I	–	–	–	Emaciated 2 weeks	3	15,000	3	2	
82c	15	+	+	I	–	–	–	Emaciated 3 weeks	2	22,000†	4	3	
53d	18	+	+	+	I	–	–	Emaciated 2 weeks	3	5,000	3	3	
82a	18	+	+	–	–	–	–	Emaciated 3 weeks	3	16,400†	4	2	
82c	21	+	+	+	I	–	–	Emaciated 1 week	2	1,150	3	1	

* Viable counts calculated from sodium hydroxide-treated homogenate shown (†). (Untreated suspension contaminated). Assessment of lesions in lung, spleen, and liver:

1 = slight macroscopic involvement
2 = moderate macroscopic involvement
3 = severe macroscopic involvement
4 = extreme macroscopic involvement

Reactions:

\+ = strongly positive
+N = additional necrosis
I = weakly positive (intermediate)
– = negative

Influence of Tuberculin Treatment on the Progress of Tuberculosis in Intranasally Infected Guinea Pigs and Mice

Guinea pigs: Twenty-six colored male guinea pigs, weighing approximately 350 gm. each, were infected intranasally with 750 tubercle bacilli. Sixteen were treated with OT from the time of infection, while the rest were retained as allergic controls. Five of the treated group received OT for six weeks only, which marked the end of the first comparison of normal and treated animals. Thereafter, these 5 animals were tested each week until the effects of the tuberculin treatment were overcome and they showed a reaction to 1:50 OT. This was a slow and variable process, requiring eight weeks in one animal and ten weeks in 2 other animals. At this stage it was decided to terminate the experiment, even though the 2 remaining animals in the group were then showing only minimal skin sensitivity to 1:50 OT.

The results of comparing hypersensitive and tuberculin-treated guinea pigs after four and six weeks, as well as temporarily treated animals fourteen and six-

TABLE 2

THE INFLUENCE OF PERMANENT AND TEMPORARY TUBERCULIN TREATMENT ON THE COURSE OF TUBERCULOSIS IN GUINEA PIGS INFECTED INTRANASALLY WITH 750 BACILLI (H37Rv)

Series	Weeks Since Infection	Tuberculin Test in Preceding Weeks							Lung Examination					Gross Involvement	
									Lobes		Nodes (Hilar)		Viable Count Lobes × 10^6		
		6	5	4	3	2	1	When Killed	Weight (Normal = 3.80 gm.)	Per Cent Increase	Weight (Normal = 1.10 gm.)	Per Cent Increase		Spleen	Liver
Hypersensitive															
H1	4	·	·	·	–	I	+N	+N	4.34	14	2.00	133	50	0	0
H2	4	·	·	·	–	–	+N	+	6.00	58	2.06	142	650	1	0
H3	6	·	–	–	+	+	+N	+	6.15	62	3.35	204	442	3	2
H4	6	·	–	–	I	+	+	+	6.20	63	3.25	187	400	2	2
H5	6	·	–	–	+	+	+N	+	6.76	78	4.61	318	875	4	2
H6	6	·	–	–	–	+	+	+	3.85	1.3	1.30	18	40	0	0
H7	14	+	+	+	+	+	+	+	5.80	55	4.50	309	140	2	2
H8	16	+N	+N	+	+N	+	+	I	8.00	110	6.35	480	955	4	3
H9	16	+	+N	+N	+	+N	+	+	7.50	97	4.45	350	350	4	3
H10	16	+	+	I	+	+	+	+	7.80	105	5.62	410	320	4	2
Treated Permanently															
P1	4	·	·	·	–	–	–	–	3.80	2.4	1.70	55	83	0	0
P2	4	·	·	·	–	–	–	–	4.53	19	1.10	0	20	0	0
P3	6	·	–	–	–	–	–	–	5.60	47	3.52	136	417	1	0
P4	6	·	–	–	–	–	–	–	4.92	30	1.30	18	420	0	0
P5	6	·	–	–	–	–	–	–	4.87	28	1.45	32	10	0	0
P6	6	·	–	–	–	–	–	–	4.24	11.6	1.29	17	410	0	0
P7	6	·	–	–	–	–	–	–	5.70	50	2.84	158	870	0	0
P8	14	–	–	–	–	–	–	–	4.17	9.8	2.54	131	160	0	0
P9	16	–	–	–	–	–	–	–	6.20	63	3.00	173	2.5	0	0
P10	16	–	–	–	–	–	–	–	6.70	76	2.00	82	85	0	0
P11	16	–	–	–	–	–	–	–	6.80	79	2.00	82	283	0	0
Treated Temporarily (6 weeks)															
T1	14	–	–	–	–	I	+	+	8.76	130	4.05	268	1,820	3	2
T2	16	–	–	–	–	I	+	I	15.10	297	2.80	155	9,500	3	4
T3	16	–	–	–	–	I	+	+	7.50	97	3.90	254	4,000	4	3
T4	16	–	–	–	–	–	–	I	5.20	37	3.20	191	31	0	0
T5	16	–	–	–	–	–	–	I	5.60	47	2.50	127	20	0	0

Series:

H = hypersensitive, (1:50 OT) receiving 1 ml. 0.3 per cent phenol saline daily.
P = permanently treated with tuberculin.
T = temporarily treated with tuberculin (6 weeks), then held until sensitive to 1:50 OT.

Tuberculin tests:

H group—1:50 OT into shaven dorsal skin each week.
P group—1:10 OT as above.
T group—1:10 OT while receiving tuberculin treatment, then 1:50 OT until skin sensitivity appeared.

Reactions:

\+ = strongly positive
+N = additional necrosis
I = weakly positive (intermediate)
– = negative

Gross involvement of liver and spleen:

1 = slight macroscopic involvement
2 = moderate macroscopic involvement
3 = severe macroscopic involvement
4 = extreme macroscopic involvement

teen weeks after infection, are given in table 2. The comparison was based on the degree of gross involvement of lungs, hilar nodes, livers, and spleens, and on the bacillary population of the lungs. In contrast to the experiments of Woodruff (4–6), the animals were not allowed to die. All were in good physical condition at the time of sacrifice except for *T*2 which showed some weight loss.

A comparison of tuberculin-sensitive and tuberculin-treated guinea pigs killed four, six, or sixteen weeks after infection revealed certain well-defined group differences. In contrast to the arrest of lesions apparent during the allergic phase in mice (2), allergic guinea pigs showed a slow, but progressive spread within and from the lungs to hilar nodes, spleens, and livers, despite the arrest of bacillary multiplication in the lungs (and presumably elsewhere) similar to that observed in mice. The treated group, however, showed a slower rate of lung involvement and little tendency toward gross dissemination of the disease from the primary site. The gross distribution of lesions in the tuberculin-treated group thus bore a striking resemblance to the allergic pattern in mice (2), viz., well-developed lung lesions, minimal hilar lymph node involvement, and no tendency toward gross involvement of other organs.

This finding agrees with the observations of Woodruff and his colleagues (4–6) who noted the absence of gross lesions in the spleens and livers of tuberculin-treated guinea pigs infected subcutaneously with 0.5 mg. of tubercle bacilli. However, massive tuberculous pneumonia of the type seen by these workers in treated animals that were permitted to die was not evident in the present animals, sacrificed while in apparently good health. Therefore, acute tuberculous pneumonia in tuberculin-treated guinea pigs must be regarded as either terminal in origin or due to excessive doses of tuberculin. In the present series the degree of lung and hilar node involvement was actually a good deal less than that in the comparable sensitive controls. An impression of this difference, which was readily apparent at autopsy, may be gained from the accompanying photograph (figure 1) and confirmed by comparing the average increases in lobe and lymph node weight for the whole series. Taking as a base line the mean weight of lobes and nodes from 20 healthy adult male guinea pigs (3.80 gm. and 1.10 gm., respectively), the average increase in weight in the sensitized group was 64 per cent (lobes) and 252 per cent (nodes). The corresponding figures in the tuberculin-treated group were 38 per cent and 82 per cent, respectively.

At no time was any significant difference apparent in the bacterial population of the lungs of sensitive and treated animals. It is concluded that guinea pigs rendered insensitive to 1:10 OT by tuberculin treatment resembled mice (2) in failing to show the rapid bacillary multiplication which characterized naturally occurring anergy to 1:50 OT. On the contrary, the severity of the disease in the tuberculin-treated guinea pigs in terms of gross lesions was less than that in tuberculin-sensitive controls, presumably due to suppression of allergic damage.

The small group of temporarily tuberculin-treated animals was included in the experiment on the mistaken assumption that treatment, like natural anergy, would lead to a massive multiplication of bacilli, which might be reversible when these animals were allowed to develop sensitivity. Although contributing little to the picture that emerged, they are included because they show that the effects

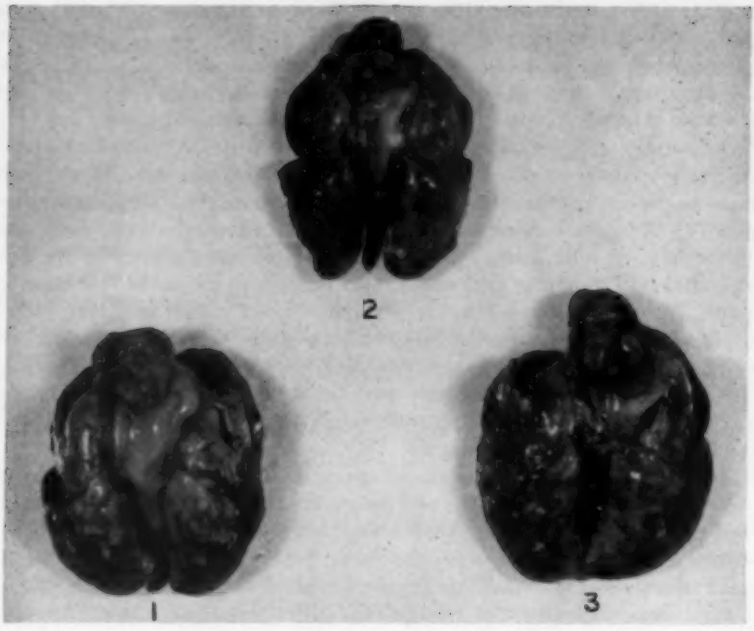

FIG. 1. Comparison of lung and lymph node involvement in sensitive and permanently and temporarily tuberculin-treated guinea pigs fourteen weeks after infection.

1. Guinea pig H7 (sensitive to 1:50 OT)
2. Guinea pig P8 (insensitive to 1:10 OT)
3. Guinea pig T1 (*see* table 3). (Insensitive to 1:10 OT, 6 weeks; then allowed to become sensitive to 1:50 OT).

Guinea Pig	Lobe Weight	Lymph Node Weight	Viable Tubercle Bacilli
	gm.	gm.	
H7	5.8	4.5	140,000
P8	4.17	2.54	160,000
T1	8.76	4.05	1,830,000

The lighter areas represent normal lung tissue.

of treatment that was continued over a period of six weeks were not readily lost. Insensitivity to 1:50 OT was maintained for eight to ten weeks after daily tuberculin injections ceased. In the 3 animals (T1, T2 and T3, table 2) in which sensitivity had become well established, allergic damage was again evident at the time of sacrifice. The hilar nodes, livers, and spleens resembled those of the hypersensitive animals in being grossly involved, and there was evidence that the lung lesions were progressing rapidly, with rising bacterial numbers. The remaining 2 animals (T4 and T5), killed when showing the first minimal skin reaction,

presented essentially the same picture as animals treated for the whole period of observation.

Mice: The observed tendency of tuberculin treatment to give rise to a mouse-type of distribution of lesions in guinea pigs, presumably by suppressing allergic inflammation, suggested that it would be profitable to study the effects of treatment on the course of tuberculosis in mice, which are much less susceptible to allergic damage.

Fifty C57 mice were infected at the age of ten weeks with approximately 30 viable units intranasally and were subjected to footpad testing at weekly intervals in the usual way. Two weeks later, well before the expected appearance of the earliest footpad reaction, a group of 20 mice was placed on a daily routine injection of 0.2 ml. of concentrated OT subcutaneously. This relatively large dose was tolerated more readily and with much less inflammatory reaction than was noted in guinea pigs.

Ten days later, 30 mice were sacrificed. These consisted of 10 mice with no acquired footpad sensitivity, 10 mice that had shown a positive reaction three days earlier, and 10 tuberculin-treated mice. Ten weeks after infection, 13 additional tuberculin-treated mice which had then received OT for seven weeks were compared with 12 hypersensitive controls, which had given a regularly positive footpad reaction for at least four weeks prior to sacrifice.

The results of these comparisons may be seen in table 3. Circumstances at the time would not permit individual examinations, so these results were de-

TABLE 3

THE INFLUENCE OF DELAYED TUBERCULIN TREATMENT ON COURSE OF TUBERCULOSIS IN C57 BLACK MICE (10 WEEKS OLD) INFECTED INTRANASALLY WITH 30 BACILLI (H37Rv)

Number of Mice	Interval Since Infection	Tuberculin Treatment	Footpad Sensitivity in Preceding Weeks							Lung Examination				Viable Counts $\times 10^8$ Pooled Lobes
										Lobes		Nodes		
			6	5	4	3	2	1	When Killed	Average Weight	Per Cent Increase*	Average Weight	Per Cent Increase*	
	weeks	*weeks*								*gm.*		*gm.*		
10	4½	Nil	·	·	·	·	−	−	−	0.17	6	0.13	15	875
10	4½	Nil	·	·	·	·	−	−	+	0.18	12.4	0.14	23	800
10	4½	1½	·	·	·	·	−	−	−	0.17	6	0.13	8	750
12	10	Nil	−	+	+	+	+	+	+	0.30	87.5	0.20	82	380
13	10	7	−	−	−	−	−	−	−	0.23	42	0.16	45	710

Tuberculin treatment: Daily dose of 0.2 ml. concentrated OT commenced 2 weeks after infection and was maintained for duration of experiment.

Footpad test: 1:25 OT, 0.03 ml. in alternate hind feet each week.

* *Normal lung lobe and node weights:*

		Lobe weight average	Node weight average
Base line 20 normal mice at 12 weeks	—	0.15 gm.	0.1 gm.
20 normal mice at 22 weeks	—	0.17 gm.	0.12 gm.
	Mean:	0.16 gm.	0.11 gm.

Reactions:

\+ = strongly positive
+N = additional necrosis
I = weakly positive
− negative

termined on the pooled organs of each group of 10 mice. The figures given therefore represent the average findings of each group. The results were remarkable mainly for their uniformity. Tuberculin treatment appeared to exert no significant effect on the course of tuberculosis in C57 mice unless it was to restrict the degree of hilar nodal involvement and the rate of progress of lung lesions to a limited extent. As found in the guinea pig, conditions simulating those observed in naturally occurring anergy were not reproduced by tuberculin treatment.

These findings suggest that an essential difference between the allergic response of the mouse and guinea pig to tuberculosis is to be found in the greater susceptibility of the guinea pig to dissemination of the infection and to gross spleen and liver involvement, a phenomenon that might be termed allergic damage. Tuberculin treatment partly counteracts the susceptibility of guinea pigs to allergic inflammation, so that treated guinea pigs tend to produce a mouse-type of response to infection.

Discussion

At the clinical level, skin hypersensitivity (allergy) is accepted as an index of the onset of acquired immunity in tuberculosis, but the subsequent relationship of allergy to the intensity and duration of the immune response has been the subject of a great deal of argument. The present results may help to eliminate at least some of the existing confusion.

Although it is not yet possible to explain why it should occur, a relatively sudden and unpredictable loss of immunity was observed in both mice and guinea pigs infected with virulent tubercle bacilli. This was associated with loss of the skin sensitivity that had been present throughout the period of specific immunity and was followed by death within a few weeks. It seems justified to conclude that this may well be the normal pattern of the terminal stages of tuberculosis. The proviso must be made that this pattern may often be upset, especially in guinea pigs and apparently also in man (3), because prematurely fatal complications are common, presumably due to allergic damage, while the level of immunity is still high.

Support for this view was obtained from a study of the rates of multiplication of bacilli and of extension of lesions in mouse lungs in relation to their tuberculin sensitivity (2). This work led to the conclusion that uninhibited multiplication of tubercle bacilli occurred under the existing experimental conditions only in animals in which allergy was not present. It also raised the question whether the same relationship might exist in other species of animals examined under appropriate conditions.

Allergy appears so quickly after infection in guinea pigs that preallergic deaths seldom occur, even when large infecting doses are employed. For this reason also, it is difficult to demonstrate rapid preallergic multiplication of bacilli. However, as an indication that it probably does occur, when guinea pigs were infected by intracutaneous inoculation (1), a steady rise in stainable bacillary numbers was observed in the skin lesions, the rise terminating after three weeks with the appearance of skin sensitivity. Thereafter the numbers became pro-

gressively fewer in the local skin lesions. To this may be added the evidence obtained from a limited number of observations on congenital tuberculosis in guinea pigs which was characterized by massive numbers of bacilli in the lesions (unpublished). Current studies (table 1) confirmed the observations of Woodruff and his colleagues (4, 5) that a terminal anergic flare occurs in the lungs of guinea pigs in the absence of prematurely fatal complications and suggest the existence of a basic mouse-type of relationship of allergy to bacterial growth in guinea pigs. This relationship, however, is frequently complicated in guinea pigs by their sensitivity to allergic damage involving vital organs. It is believed that careful handling of infected guinea pigs in the later stages of the disease will reduce the danger of premature death from rupture of a grossly diseased liver or spleen.

There is little information in the literature concerning rapid preallergic multiplication in man, although workers in this field are familiar with the enormous numbers of tubercle bacilli to be found not only in the lungs but also in spleen lesions in congenital tuberculosis. Woodruff (3) has produced evidence, however, of a terminal anergic flare in human cases, so that the mouse-type of pattern may well be applicable to man, with the same reservations as in guinea pigs regarding allergic damage.

Finally, published data leave no reason to suppose that the relationship of allergy to bacillary growth in the rabbit lung differs from that in the other species mentioned.

Thus, the evidence presented suggests the occurrence of unrestricted multiplication of tubercle bacilli in several animal species when allergy, as determined by tuberculin sensitivity, is not present.

It is believed that the initial observation of this phenomenon in mice was made possible by the use of a small infective dose of tubercle bacilli given by the intranasal route. Recent studies of mice infected intravenously with a very large dose of bacilli indicate that a proportion of such animals may show the same type of deviation from the suggested mouse pattern as was seen in some tuberculous guinea pigs (table 1), in which it was apparently related to prematurely fatal complications often involving organs other than the lungs. This may account, at least in part, for the failure of the phenomenon described here to be observed earlier.

If the above evidence for the chronologic relationship of allergy to the immune state is valid, the next logical step would be to consider the relative importance of allergy in the total immune response. Several workers have attempted to separate allergy from immunity by artificial desensitization with Old Tuberculin, but the results obtained in the present study indicate that natural anergy, with its terminal flare, is not the same state as that produced by tuberculin treatment. The results of earlier attempts to separate allergy and immunity on these grounds should therefore be interpreted with reserve.

Artificially desensitized mice and guinea pigs showed neither the fulminating infection nor the massive multiplication of bacilli characterizing natural anergy. On the contrary, immunity was strongly maintained in both species under tuberculin treatment, and the spread of lesions both within and from the original

site of infection was restricted. Although these results could probably be explained on the basis that mice are merely less sensitive than guinea pigs to allergic damage, they have been interpreted as indicating the possible existence of at least two components of allergy. These may be associated with the presence in the antigenic mosaic of *M. tuberculosis* of more than one substance capable of giving rise to allergic phenomena. One of these, with a much stronger manifestation in guinea pigs than in mice, may be the component responsible for allergic damage and may be independent of the immune response. This manifestation could be suppressed in guinea pigs by tuberculin treatment without affecting immunity in terms of the ability of the guinea pig to suppress the multiplication of tubercle bacilli in the lungs. Tuberculin treatment in the mouse, presumably due to the relative insensitivity of this species to allergic damage, produced no dramatic change in the disease.

The other component of allergy, which was equally manifest in mice and guinea pigs, is believed to be inseparable from that part of the immune response associated with specific enhancement of the cellular defenses. The disappearance of this component when natural anergy followed allergy in previously reacting mice and guinea pigs marked the end of the immune phase in both species. Tuberculin treatment failed to interfere with the systemic manifestations of this component of allergy although it did suppress the sensitivity of the skin to small volumes of tuberculin, as would be expected in animals whose tissues are assumed to be already full of tuberculin.

On first appraisal, the results obtained here appear to disagree with those of more extensive studies by Woodruff and his colleagues (4–6), but it should be noted that important differences existed in the experimental details. These include size of infecting dose, route of infection, and amount of tuberculin injected daily, and—of greater importance—the fact that the present results were obtained from animals sacrificed while in apparently good health instead of being allowed to die. It is believed that the massive pneumonia and enormous bacillary population observed by these workers in heavily infected animals that died after prolonged tuberculin treatment were related either to a superimposed natural anergy, masked by the suppression of skin sensitivity, or to the toxic effects of large doses of tuberculin swamping the immunity mechanism. Follis (7) showed that large doses of glycerin could produce a similar effect in a proportion of allergic guinea pigs not receiving tuberculin.

These findings seem to enhance the possibility of stimulating immunity against tuberculosis dissociated from the harmful effects of allergy, but the prospect of producing immunity completely divorced from all allergic phenomena appears to be remote. This concept derives from the belief that the component of allergy coexisting with immunity is merely a manifestation of a specific change in the cellular defenses, probably dependent on an antibody-phagocyte complex and, therefore, an important part of the immune response.

A great deal of work remains to be done to assess the significance of the allergic phase in immunity against tuberculosis, but there is an increasing body of evidence pointing to an incomplete role of allergy per se. Recent advances in

our knowledge, notably the immunization of experimental animals with fractions of tubercle bacilli (10–12), and the passive transfer of delayed hypersensitivity with an alpha globulin from monocytes (Jeter, personal communication), together with the specific nature of the immunity, may be interpreted as indicative of the participation of humoral, as well as cellular factors, in the immune response to tuberculosis. The work of Lurie (13) and of Fong and his associates (14) may also be interpreted as indicating that specific protective antibodies mediate the defenses of the monocytes. Their distribution in the body, in addition to their probable attachment to monocytes, remains to be determined. The observations of Brieger, Fell, and Smith (15) on monocytes from tuberculous rabbits would support the theory presented above. Monocytes taken from these animals contained few or no stainable bacilli, indicating successful suppression of multiplication by the allergic host. Within a few days of removal from the immune environment to tissue culture, presumably still sensitive to tuberculin, these cells were packed full of acid-fast bacilli, suggesting that the monocytes, the main vehicle of passive transfer of allergy, were incapable of maintaining per se the degree of suppression possible within the animal body.

The presence or absence of complement in tissue-culture studies may well explain the confusion that has arisen from attempts to demonstrate the role of specific antibody in enhancing the antituberculous activity of phagocytic cells in this situation.

The relative importance of cellular and humoral factors in immunity against tuberculosis could be tested experimentally. It is not difficult to transfer allergy passively from sensitized to normal animals by inoculating washed monocytes. It should be possible to determine whether this type of passive transfer, either alone or when supported by serum components and maintained by repeated inoculations, would at the same time confer passive immunity.

If immunity in this disease can be shown to differ only quantitatively and not qualitatively from the normal pattern seen in other diseases which are now controlled successfully with killed vaccines or antigenic fractions of microorganisms, the future of immunization may be more hopeful than has been apparent in recent years.

Summary

The previously reported correlation between footpad sensitivity to tuberculin and the ability of C57 mice to survive intranasal tuberculous infection (1, 2) suggested that this might be the pattern of tuberculosis in other animal species examined under the appropriate conditions.

Guinea pigs, which are not subject to preallergic deaths owing to an earlier appearance of allergy in this species compared with mice, were infected intranasally with a small number of tubercle bacilli. About half the animals died while they were tuberculin reactors, but all of these were in good physical condition with moderate lesions and restricted numbers of bacilli, and death could usually be accounted for by some prematurely fatal complication. The remainder, dying after the onset of anergy, showed both the terminal emacia-

tion and the terminal flare in the number of bacilli in the lungs observed in mice.

Desensitization of guinea pigs and mice by repeated injections with OT designed to saturate the tissues failed to reproduce the state of natural anergy. Both lung lesions and bacillary numbers compared favorably with those which occurred in tuberculin-sensitive controls.

In desensitized guinea pigs, however, the spread of disease from the lungs was dramatically restricted so that the distribution was reminiscent of that seen in mouse tuberculosis. It is postulated that tuberculin treatment in guinea pigs minimizes allergic damage. This restriction was slight in tuberculin-treated mice, which is presumed to be due to the relative insusceptibility of this species to allergic damage.

These results suggest the existence of two separate hypersensitive states in tuberculosis, in both of which intracutaneous tuberculin causes reactions, and reactivity to tuberculin is not detectable when the tissues are in constant contact with tuberculin. The systemic effects of one of these states, presumably associated with the phenomenon of allergic damage, were suppressed by tuberculin treatment. Those of the other state, which may be related to a specific immune change in phagocytic cells, were not suppressed by tuberculin.

Allergy appears to be inadequate per se to account for all aspects of the immune response to tuberculosis.

Sumario

Inmunidad, Anergia Natural y Desensibilizacion Artificial en la Tuberculosis Experimental

La correlación ya comunicada entre la sensibilidad de la planta del pie a la tuberculina y la capacidad de C57 ratones para sobrevivir la infección tuberculosa intranasal (1, 2) sugirió que éste podría ser el patrón de la tuberculosis en otras especies animales estudiadas en condiciones apropiadas.

Se infectó intranasalmente con una pequeña cantidad de bacilos tuberculosos a cobayos que, comparados con los ratones, no están expuestos a muertes prealérgicas debido a aparición más temprana de la alergia en esta especie. Aproximadamente la mitad de los animales falleció mientras eran reactores a la tuberculina, pero todos ellos se encontraban en buen estado físico con lesiones moderadas y limitadas cantidades de bacilos, de modo que por lo general no podía imputarse la muerte más que a alguna complicación prematuramente letal. Los demás, que murieron después de la iniciación de anergia, revelaron tanto la emaciación terminal como la exaltación terminal del número de bacilos observado en los pulmones de los ratones.

La desensibilización de los cobayos y los ratones con inyecciones repetidas de T. A., destinadas a saturar los tejidos, no reprodujo el estado de anergia natural. Tanto las lesiones pulmonares como las cantidades de bacilos se compararon favorablemente con las observadas en los testigos tuberculinosensibles.

Sin embargo, en los cobayos desensibilizados, la difusión de la enfermedad desde los pulmones se limitó dramáticamente, de modo que la distribución hacía recordar la observada en la tuberculosis murina. Presupónese que la tuberculinoterapia en los cobayos reduce al mínimo la lesión alérgica. Esta limitación fué ligera en los ratones tratados con tuberculina, lo cual se debe presuntamente a la relativa insusceptibilidad de esta especie a la lesión alérgica.

Estos resultados sugieren la existencia de dos distintos estados hipersensibles en la tuberculosis, en ambos de los cuales la tuberculina intracutánea provoca reacciones y en

que no puede descubrirse la reactividad a la tuberculina cuando los tejidos se hallan en constante contacto con la tuberculina. Los efectos constitucionales de uno de estos estados, asociado presuntamente con el fenómeno de la lesión alérgica, fueron suprimidos por la tuberculinoterapia. Los del otro estado, que tal vez se relacionen con una inmuni-alteración específica de los fagocitos, no fueron suprimidos por la tuberculina.

La alergia parece inadecuada por sí sola para explicar todas las fases de la inmuni-reacción a la tuberculina.

Resume

Immunité, anergie naturelle et désensibilisation artificielle dans la tuberculose expérimentale

La corrélation précédemment rapportée de l'allergie tuberculinique au niveau de la patte de l'animal et la capacité de survie à l'inoculation tuberculeuse intra-nasale de C57 souris (1, 2), a conduit à supposer que ceci pouvait correspondre aux modalités de la tuberculose dans d'autres espèces animales examinées dans des conditions appropriées.

Les cobayes plus résistants dans la phase pré-allergique, par comparaison avec les souris, ont été inoculés par voie nasale avec un petit nombre de bacilles tuberculeux. Environ la moitié des animaux a succombé durant la phase de réaction positive à la tuberculine. Toutefois, tous ces animaux étaient en bon état physique, les lésions étaient de degré modéré et le nombre de bacille réduit; la mort pouvait habituellement être expliquée par une complication prématurée fatale. Le reste, où la mort est survenue après l'apparition de l'anergie, a présenté l'émaciation terminale et la brusque augmentation terminale des bacilles pulmonaires qui sont observées chez la souris.

La désensibilisation des cobayes et des souris par les injections répétées de tuberculine de Koch (OT), faites dans le but de saturer les tissus, n'a pas réussi à reproduire l'état d'anergie naturelle. Le degré des lésions pulmonaires et le nombre de bacille se comparaient favorablement avec les valeurs enregistrées dans le groupe de contrôle tuberculino-sensible.

Néanmoins, chez le cobaye désensibilisé, l'extension de l'affection à partir du poumon était limitée de façon spectaculaire de telle sorte que la répartition rappelait celle observée dans la tuberculose de la souris. L'auteur considère que, chez le cobaye, le traitement par la tuberculine minimise les altérations d'ordre allergique. Cette réduction était insignifiante chez la souris traitée par la tuberculine, ce qui est supposé en relation avec la non-susceptibilité de cette espèce aux lésions allergiques.

Ces résultats suggèrent l'existence de deux états allergiques distincts dans la tuberculose et, dans chacun d'eux, a tuberculine intradermique détermine des réactions; cette réactivité à la tuberculine n'étant pas décelable lorsque les tissus sont en contract permanent avec la tuberculine. Les effets organiques de l'un de ces états, attribués au phénomène de l'altération d'ordre allergique, ont été supprimés par le traitement à la tuberculine. Ceux de l'autre état, qui peuvent attribués à une modification des cellules phagocytes sous l'influence d'une immunité spécifique, n'étaient pas supprimés par la tuberculine.

L'allergie à elle seule ne parait pas suffire à expliquer tous les aspects de la réaction d'immunité à la tuberculose.

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CHEMOTHERAPY OF TUBERCULOSIS[1]

III. Verazide in the Treatment of Pulmonary Tuberculosis

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Introduction

Verazide is 3:4-dimethoxybenzal *iso*nicotinyl hydrazone and has been previously described by the present writers (1, 2). On the evidence available from the laboratory studies, it was considered worth while to investigate its potential value in the treatment of pulmonary tuberculosis. Already hydrazones of isoniazid have been used clinically; for example, 3-methoxy-4-hydroxybenzal hydrazone (Phtivazid®) is extensively used in the Soviet Union (3) and *o*-hydroxybenzal hydrazone (Nupasal®) has been clinically investigated in England (4).

The present study aimed to establish not so much the therapeutic efficiency of verazide, which, on the accumulated *in vivo* evidence, seemed self-evident, but to determine whether verazide was chemotherapeutically better than isoniazid. This seemed a legitimate aim, since there are no indications for using verazide in preference to isoniazid except on the grounds that its therapeutic index in animal experiments is measurably better than that of the parent substance, isoniazid. It had already been established, for example, that tubercle bacilli resistant to isoniazid were equally resistant to verazide and vice versa (1). However, animal experiments and chronic toxicity tests in humans using enteric-coated tablets indicated that verazide was markedly less toxic than isoniazid (2), and this and other characteristics encouraged the writers to examine its value in human tuberculosis.

From the results reported herein, the conclusion has been reached that, while verazide is therapeutically active in all stages of pulmonary tuberculosis, the response it effects is indistinguishable from that seen with isoniazid.

Plan of Investigation

Selection of patients: The clinical trial was conducted simultaneously in three different hospitals: Heatherton Sanatorium, Melbourne; Gresswell Sanatorium, Melbourne; and Randwick Chest Hospital, Sydney. Patients were admitted to the trial on a purely random basis, that is, every alternate patient who had not previously been treated chemotherapeutically was given verazide therapy. The other patients were treated with isoniazid. The cases were classified in accordance with the 1950 classification of the National Tuberculosis Association after being allocated to the verazide or isoniazid group. For ethical reasons all patients received multiple-drug therapy, the supporting drug over the period of observation being streptomycin. It was a condition of the trial that each group received exactly the same streptomycin therapy, although the dosage of streptomycin varied slightly in the different hospitals.

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Assessment of treatment: The assessment of therapeutic response was based almost entirely on roentgenographic and bacteriologic findings; but other criteria, such as gain in weight, disappearance of sputum, temperature, and clinical findings, were also taken into account. However, these did not provide any information which was not revealed by the more objective criteria of roentgenographic and bacteriologic findings. Each patient was fully investigated on admission and reinvestigated at three- and six-month intervals. For the purpose of this study the information sought was available at the end of three months, although most of the patients continued to receive treatment for six or more months.

The roentgenographic data were obtained by reading routine films, and tomograms were seldom used. The films were examined by clinical specialists in each of the hospitals, and the assessment was made by reference to the original films. The results were graded as improved, worse, or no change. If improvement was recorded, this was reported as:

+++ indicating very marked improvement, better than expected result
++ indicating marked improvement, a good but expected result
+ indicating moderate improvement, a fair but expected result

The bacteriologic examinations were carried out more intensively than is usual in routine diagnostic work, in that three specimens of sputum were examined by microscopy and culture on three successive days, using four Löwenstein slants per specimen and incubating for ten weeks at 37°C. Gastric contents were similarly examined by culture when sputum was not available, and drug-susceptibility tests, by direct or indirect technique, were made on all isolates. The bacteriologists were given no indication of the treatment.

Number of patients: In all, 154 patients were admitted to the trial: 79 were treated with verazide and streptomycin and 75 received isoniazid and the same dosage of streptomycin. Apart from this chemotherapy, no other active treatment was instituted. All patients went through the regimen of relative bed rest with only bathroom and toilet privileges allowed during the first three months, after which moderate activity was allowed, gradually increasing up to the six-month period.

Chemotherapy: Verazide was given in a dose equivalent to 20 mg. per kg. daily by mouth as enteric-coated tablets (shellac-treated) in divided doses twice daily after meals. Some patients at the commencement of the trial were given verazide in uncoated tablets, but this was discontinued for reasons given below.

The isoniazid group in the Heatherton and Gresswell sanatoriums received isoniazid, 8 mg. per kg. daily in two divided doses. All patients in these hospitals also received 1 gm. of streptomycin on alternate days over the period of the trial.

The patients who were treated in the Randwick Chest Hospital received verazide, 20 mg. per kg., as described above, but were given 1 gm. of streptomycin daily for six weeks and thereafter three times a week. The isoniazid-control subjects in this hospital received 300 mg. daily irrespective of their weight, together with the same dose of streptomycin.

Results

Roentgenographic findings: The roentgenographic findings from the three hospitals are recorded in table 1 and are summarized in table 2. It will readily be seen that the two groups of patients were well matched with respect to the severity of their disease and that the response to treatment in each group followed a similar pattern. This is particularly well demonstrated in table 2, which shows that 83 per cent of the patients in the isoniazid-control group showed roentgenographic improvement, and in the verazide group 82 per cent of the patients showed improvement. The stage of the disease did not significantly alter the favorable response to either drug; the patients with moderately advanced disease seemed to respond better to isoniazid, but the reverse held for the

TABLE 1

ROENTGENOGRAPHIC CHANGES AFTER THREE MONTHS' CHEMOTHERAPY

Hospital	Chemotherapy (*see* text)	Number of Patients	Classification of Patients		Response of Patients			Degree of Improvement (*see* text)		
			Degree	Number	Worse	No Change	Improved	+	++	+++
Gresswell Sanatorium	Isoniazid	30	Minimal	6	0	2	4	4	0	0
			Moderate	10	0	0	10	6	4	0
			Advanced	14	1	2	11	5	4	2
Heatherton Sanatorium	Isoniazid	34	Minimal	7	0	1	6	5	0	1
			Moderate	13	1	0	12	5	5	2
			Advanced	14	0	2	12	3	8	1
Randwick Chest Hospital	Isoniazid	11	Minimal	3	0	1	2	2	0	0
			Moderate	3	0	0	3	3	0	0
			Advanced	5	1	2	2	2	0	0
Gresswell Sanatorium	Verazide	34	Minimal	4	0	0	4	4	0	0
			Moderate	17	1	1	15	9	5	1
			Advanced	13	0	0	13	7	5	1
Heatherton Sanatorium	Verazide	32	Minimal	8	0	2	6	3	3	0
			Moderate	12	0	3	9	6	2	1
			Advanced	12	2	1	9	3	3	3
Randwick Chest Hospital	Verazide	13	Minimal	1	0	1	0	0	0	0
			Moderate	2	0	1	1	0	1	0
			Advanced	10	0	2	8	7	1	0

TABLE 2

ROENTGENOGRAPHIC CHANGES AFTER THREE MONTHS' CHEMOTHERAPY

(Summary of Table 1)

Chemotherapy	Number of Patients	Classification of Patients		Patients Showing Improvement		Patients Showing No Improvement or Deterioration	
		Degree	Number	Number	Per Cent	Number	Per Cent
Isoniazid	75	Minimal	16	12	75	4	15
		Moderate	26	25	96	1	4
		Advanced	33	25	76	8	24
		Combined	75	62	83	13	17
Verazide	79	Minimal	13	10	77	3	23
		Moderate	31	25	81	6	19
		Advanced	35	30	86	5	14
		Combined	79	65	82	14	18

patients with far advanced disease. Calculating the response on the basis of a 2-plus or 3-plus roentgenographic improvement, it was found that 35 per cent of the isoniazid-treated and 33 per cent of the verazide-treated patients fell into this category. It is apparent that no distinction can be drawn between the verazide and isoniazid groups of patients on the roentgenographic evidence, irrespective of the manner in which the evidence is used.

The results with considerably fewer patients after six months' treatment showed exactly the same trend. Other clinical criteria, such as gain in weight, disappearance of sputum, and general feeling of well-being, supported the roentgenographic data.

Bacteriologic findings: The bacteriologic results are summarized in tables 3 and 4. In table 3 the patients were classified, as at the commencement of treatment, into two broad groups, those with sputum and those without, and each group was further subdivided as indicated in the table. It will be seen that the reversal of infectiousness, as evidenced by cultures negative for tubercle bacilli, was highly successful with both drugs in all but the first group, which contained those patients with sputum positive by microscopy and culture. Some difference can be detected between the two drugs in this group in favor of verazide: whereas the sputum of 63 per cent of the patients on verazide therapy became noninfectious, only 52 per cent of the isoniazid-treated patients exhibited the same type of response. However, this difference is probably not significant. For, on reassembling the data (as in table 4), it can be shown that 74 per cent of patients from whom *M. tuberculosis* was isolated, either from sputum or gastric lavage, after three months' treatment with either verazide or isoniazid were nonexcretors. These findings closely parallel the roentgenographic results and indicate quite clearly that the clinical response to verazide and isoniazid is indistinguishable.

Toxic reactions: In none of the patients taking isoniazid were any toxic manifestations observed. On the other hand, toxic reactions to verazide were noted, depending on the form in which the drug was administered. These reactions will be briefly reported.

At the beginning of the present trial it was decided to give verazide as plain tablets since in an earlier study (2) it was found that the combined serum concentration of isoniazid and verazide was higher than that obtained with the enteric-coated tablets. It soon became obvious, however, that some patients tolerated the plain tablets very poorly. The toxic symptoms were mainly those of sleepiness, giddiness, slurred speech, extreme drowsiness, and nausea. In fact, they closely mimicked symptoms of drunkenness in varying degrees and usually appeared within a week of commencement of treatment. On withdrawing the drug the symptoms disappeared and did not reappear when a placebo was given. However, when the same patients were given verazide in enteric-coated tablets, none of these toxic reactions were observed. It appeared, therefore, that the release of relatively large amounts of veratric aldehyde (approximately 4 mg. per kg. daily) during partial hydrolysis in gastric juice was responsible for

TABLE 3

BACTERIOLOGIC CHANGES AFTER THREE MONTHS' CHEMOTHERAPY

Bacteriologic Status at Commencement	Chemotherapy	Number of Patients	Examined Bacteriologic Status of Patients After Three Months		
			Smear −ve Culture −ve	Smear −ve Culture +ve	Smear +ve Culture +ve
Sputum					
Smear—positive	Isoniazid	27	14	7	6
Culture—positive	Verazide	35	22	9	4
Sputum					
Smear—negative	Isoniazid	19	18	1	0
Culture—positive	Verazide	9	9	0	0
No sputum					
Gastric culture*—positive	Isoniazid	8	8	0	0
	Verazide	9	8	1	0
No sputum					
Gastric culture—negative	Isoniazid	17	17	0	0
	Verazide	18	18	0	0

* Culture from gastric lavage.

TABLE 4

BACTERIOLOGIC CHANGES AFTER THREE MONTHS' CHEMOTHERAPY

Chemotherapy	Number of Patients from Whom *M. tuberculosis* Was Isolated Before Treatment	Number of Patients from Whom *M. tuberculosis* Could Not Be Isolated after Three Months' Treatment
Isoniazid	54	40 (74%)
Verazide	53	39 (74%)

the syndrome. In view of these observations, the trial proceeded with the use of enteric-coated tablets.

Of the 75 patients taking the enteric-coated tablets, 4 developed peripheral neuritis; one patient was a chronic alcoholic, and another was suffering from a peripheral vascular disease. Nevertheless, the signs and symptoms were unequivocal and unexpected in spite of the high dosage of verazide, equivalent to 10 mg. of isoniazid per kg. daily. In the present writers' previous paper (2), free isoniazid was sought in the serum of subjects taking isolated doses of enteric-coated verazide but none was detected. These findings and the extraordinarily close similarity of the clinical, roentgenographic, and bacteriologic response in the two groups of patients led to investigation of the stability of verazide and other hydrazones in blood and other tissues. These findings will be reported separately (5), but it can be stated here that verazide and other hydrazones of isoniazid are hydrolyzed *in vivo* to isoniazid.

Discussion

The object of this investigation was to compare the chemotherapeutic efficiency of verazide with that of isoniazid. To this end, 154 patients suffering from pulmonary tuberculosis were selected for study, 75 of whom were treated with isoniazid and 79 with verazide. Each group of patients received streptomycin, in addition.

The results were assessed after three-months' therapy and were sufficiently clear-cut on clinical, roentgenographic, and bacteriologic criteria to enable the writers to form a definitive opinion. It was found that the chemotherapeutic response to verazide and isoniazid was indistinguishable (tables 1–4). For example, 82 per cent of the patients on verazide therapy showed roentgenographic improvement after three months and 83 per cent of the isoniazid-control patients showed similar improvement (table 2). The response of the patients was not affected by the stage of the disease or by the hospital in which they were treated (table 1). The bacteriologic findings closely paralleled the above in that 74 per cent of patients with "open" lesions in each group were rendered noninfectious after three months of therapy (table 4). That these favorable results could be attributed to verazide was unexpectedly shown in one patient whose advanced pulmonary lesion failed to show improvement after two months' treatment. It was then discovered that this patient had secretly discarded his daily dose of verazide tablets but had continued to receive the normal dose of streptomycin by injection.

While no distinction can be drawn between the two regimens, it might be said that, on a dosage comparison, verazide is less active than isoniazid since the former was used in a dose of 20 mg. per kg. and the latter at 8 mg. per kg. This is probably not so. Late in the trial it was observed that unchanged verazide tablets could be detected in the stools of patients, and it was apparent that the enteric coating resulted in an irregular absorption of the drug in many patients. In spite of this uncontrolled variable, the results were still equal to those of isoniazid.

The toxicity of verazide (and other hydrazones of isoniazid) has been previously determined in animals, and there is little doubt that the drug is much less toxic than isoniazid. A chronic toxicity trial, using enteric-coated tablets, produced no abnormal reactions. In the present study, evidence of central nervous system intoxication was obtained when plain uncoated tablets were used. The manifestations were drowsiness, slurred speech, and sometimes blurred vision and nausea. On the other hand, when the drug was administered in enteric-coated tablets, none of these symptons were observed. It appeared that these toxic effects were due to the rapid absorption of veratric aldehyde released during partial acid hydrolysis of verazide in the gastric juice.

While the enteric-coated verazide tablets in a dose of 20 mg. per kg. of body weight did not induce the toxic effects mentioned above, 4 elderly patients developed peripheral neuritis while taking the drug. This suggested that verazide was being hydrolyzed in the blood and tissues to isoniazid. Accordingly, using a chemical technique modified from the method described previously (2),

it was possible to show that verazide is hydrolyzed in blood and tissues to isoniazid. These findings, which will be reported separately (5), offer an explanation for the strikingly close clinical response observed in this trial.

In conclusion, the present clinical findings indicate that the high antituberculous activity of verazide can be attributed to the release of isoniazid *in vivo*.

This conclusion confirms the view expressed by Allan, McNaught, and Lees (4) who suggested that the activity of *o*-hydroxybenzal *iso*nicotinyl hydrazone (Nupasal 213) was due to isoniazid. It seems justifiable to assume on the evidence reported here and unpublished work in these laboratories that the hydrazones of isoniazid which have been examined clinically owe their activity to the liberation of isoniazid in the tissues of the host.

Summary

A clinical trial of verazide has been conducted in which the response in two groups of patients suffering from pulmonary tuberculosis has been studied over a period of three months. One group of 75 patients received isoniazid and streptomycin therapy and the other group of 79 was given verazide and streptomycin. The results, assessed on clinical, roentgenographic, and bacteriologic criteria, showed that no distinction could be drawn between verazide and isoniazid. However, verazide, when given in plain tablets, exhibited toxic reactions not observed with isoniazid. These effects were attributed to the absorption of veratric aldehyde liberated by acid hydrolysis in gastric juice. On the evidence available, no chemotherapeutic or pharmacologic advantage can be claimed for verazide, when compared with isoniazid, for the treatment of pulmonary tuberculosis.

Sumario

Quimioterapia de la Tuberculosis. III. La Veracida en el Tratamiento de la Tuberculosis Pulmonar

En esta prueba clínica de la veracida se ha estudiado durante un período de tres meses la respuesta en dos grupos de enfermos que padecían de tuberculosis pulmonar. Un grupo de 75 sujetos recibió isoniacido- y estreptomicino-terapia y al otro grupo de 79 se le administró veracida y estreptomicina. Los resultados, justipreciados a base de pautas clínicas, roentgenográficas y bacteriológicas, demostraron que no cabía trazar distinción alguna entre la veracida y la isoniacida. Sin embargo, la veracida, administrada en pastillas corrientes, manifestó reacciones tóxicas que no se observaron con la isoniacida. Estos efectos se atribuyeron a la absorción del aldelhido veriátrico liberado por la hidrólisis ácida en el jugo gástrico. A base de los datos disponibles, no puede reclamarse ninguna ventaja quimioterapéutica o farmacológica para la veracida, comparada con la isoniacida, en el tratamiento de la tuberculosis pulmonar.

Resume

Chimiothérapie de la tuberculose. III. La Vérazide dans le traitement de la tuberculose pulmonaire

Il a été effectué un essai clinique de la vérazide, dans lequel la réponse au médicament de deux groupes de malades atteints de tuberculose pulmonaire a été étudiée pendant une période de trois mois. Un groupe de 75 malades a reçu un traitement par l'isoniazide et la streptomycine; un autre groupe de 79 malades a reçu la vérazide et la streptomycine. Les résultats, évalués selon les critères cliniques, radiologiques et bactériologiques, ont montré

qu'il n'était pas possible d'établir une distinction entre ceux de la vérazide ou de l'isoniazide. Toutefois, la vérazide, lorsqu'elle était administrée en comprimés simples, provoquait des réactions toxiques non observées avec l'isoniazide. Ces effets ont été attribués à l'absorption de l'aldéhyde vératrique libérée par hydrolyse acide dans le suc gastrique. D'après les données disponibles on ne peut attribuer aucun avantage chimiothérapeutique ou pharmacologique à la vérazide par rapport à l'isoniazide dans le traitement de la tuberculose pulmonaire.

Acknowledgments

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PRODUCTION OF ANTI-LUNG SUBSTANCES IN RABBITS BY HOMOLOGOUS TUBERCULOUS TISSUE ANTIGENS[1, 2]

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INTRODUCTION

The existence or coexistence of auto-immunologic disease in various clinical states of man and animals has been established beyond doubt and, indeed, it has been suggested by Cavelti (1) that these phenomena may represent a general principle in many pathologic conditions.

Although the precise mechanisms by which these phenomena are initiated are probably quite variable, the following theoretical generalizations warrant mention: On the basis of experimental immunologic facts, foreign proteins from microorganisms, toxins, and the like, may combine with normal or altered tissue to form new antigenic complexes which in turn can stimulate the production of antibodies with specificities directed to the host tissue. The pathology resulting from an infection or other process may sufficiently alter host tissue so that the clearing organs of the body subsequently recognize it as foreign and respond with the production of specific antibodies.

Most studies dealing with the production and significance of organ antibodies have been conducted with heterologous systems, and only a very few have been undertaken to determine the possible significance of these substances in perpetuating or complicating tuberculous disease. Frick (2) produced complement-fixing lung antibodies in rats following injection of homologous lung tissue incorporated in Freund's adjuvant containing either tubercle bacilli or Old Tuberculin. In certain instances, histologic changes, interpreted as sterile pneumonia, were observed.

Using alcoholic extracts of various tissues as antigens, Fischer (3) examined human tuberculous serums in an attempt to investigate the possible existence of an auto-immune phenomenon. However, this writer was uncertain whether the frequency of positive reactions noted with the lung tissue antigen was due to auto-antibodies or to "serum lability."

Because of the interest of the present writers in the possible role of organ antibodies in tuberculosis, and anti-lung substances in particular, an investigation was undertaken to determine which factors might influence the production of such antibodies in the same species. In experiments reported here, the rabbit was selected as the test animal inasmuch as this animal is also susceptible to tuberculous disease.

MATERIALS AND METHODS

Antigens: Lungs from healthy or tuberculous rabbits were minced with sufficient unbuffered saline to effect a 1:20 suspension by weight. The suspensions were subjected to

[1] From the Department of Bacteriology, The Ohio State University, Columbus 10, Ohio.

[2] This study was supported in part by a grant from the Ohio Tuberculosis and Health Association.

homogenization in a VirTis 45 homogenizer for five minutes. The methods of administering these antigens to rabbits are dealt with in the section on results.

The normal lung tissue antigen employed for serologic procedures was further treated with ultrasonic vibrations for six minutes, using a Rich Roth ultrasonic generator (model 400) coupled to a Brush electronic hemispherical transducer (400 kc) at a power setting of 6 (250–300 v). Care was exercised to maintain the temperature of the suspension below 37°C. After centrifugation in a Servall supercentrifuge, the supernatant fluid, representing the serologic test antigen, was stored at −20°C. Tissue antigens from other organs were prepared similarly.

Serums: Serums were inactivated at 56°C. for thirty minutes prior to being tested for complement-fixing antibodies with the ultrasound-treated normal lung antigen. Since the complement-fixing demonstrations with these serums were attributable, in part, to the normal tissue antibodies described by Kidd and Friedewald (4), it was necessary to further inactivate these serums at 65°C. for twenty-five minutes to establish the level of immune antibodies. The aforementioned naturally occurring antibodies are destroyed at this temperature.

Serologic procedures: For all complement-fixation tests, a modified Kolmer technique, using two full units of complement contained in 1.0 ml., two units of hemolysin (0.25 ml.), and a 3.0 per cent suspension of sheep erythrocytes (0.25 ml.), was employed. One-tenth milliliter of the test serum dilution, 0.25 ml. of the antigen, and two full units of complement were mixed and incubated overnight in the cold, after which they were warmed, the indicator system was added, and the mixture was incubated at 37°C. for thirty minutes.

The antigen used for measuring the hemagglutinating and hemolytic antibodies to tuberculopolysaccharides consisted of sheep erythrocytes sensitized with Old Tuberculin (OT).[3] The details of the testing procedure have been reported previously (5).

Tissue powders (adsorbents): Adsorbing agents were prepared from normal tissues. Five grams (moist weight) of the desired rabbit tissue were homogenized in the VirTis 45 homogenizer and the sediment, recovered by centrifugation, was extracted with 30.0 ml. of acetone for five minutes, recentrifuged, placed on filter paper, and dried for twenty minutes in a vacuum desiccator. The dried sediment was ground in a mortar and stored at room temperature until needed.

Results

Production of anti-lung substances: Two groups of rabbits were employed in the first study. Five rabbits comprising the control group received a total of 25.0 ml. of normal lung suspension intraperitoneally. Each injection consisted of 5.0 ml., the first 3 at three-day intervals; the next, after five days; and the last, three days later. Eight other animals were injected with tuberculous lung suspensions in a similar manner. All animals were bled by cardiac puncture at weekly intervals.

After inactivating the normal tissue antibodies, all serums, including preinjection samples, were examined for anti-lung substances by the complement-fixation test. The results, recorded as titers, are shown in table 1.

In no instance did the serums from the 5 control animals exhibit any immune antibodies, yet the serums from rabbits which had received infected lung demonstrated marked serologic activity. It may be noted that the complement-fixation titers of serums from rabbits *6* to *9* were higher in general than were those obtained from the serums of rabbits *10* to *13*. This may be explained by the fact that the two groups of animals received different infected lung suspensions.

The same serums also were tested for tuberculous antibodies with OT-sensi-

[3] Old Tuberculin (4 times International Standard) was generously supplied by W. S. Hammond, Lederle Laboratories Division, American Cyanamid Corporation, Pearl River, New York.

tized sheep erythrocytes. The hemagglutination and hemolytic titers, tabulated in table 2, more or less conform to the patterns frequently found in rabbits injected with mycobacteria (6).

Soluble complexes: In carrying out the complement-fixation titrations, it was noted on occasion that certain serums were highly "anti-complementary." The possibility existed that these serums may have contained antibodies which formed soluble circulating complexes with lung antigen. However, attempts to precipitate such complexes by the alkalinization technique of Sternberger and associates (7) were unsuccessful, but serologic evidence for the presence of lung antigen in these serums was obtained.

TABLE 1

PRODUCTION OF ANTI-LUNG SUBSTANCES

Serums	Complement-Fixation Titers of Lung Antibodies (weeks)						
	0	1	2	3	4	5	6
1–5 (normal lung)	—	—	—	—	—	—	—
6	—	2*	32	64	32	...	...
7	—	2	8	4	8	...	...
8	—	2	8	16	32	4	...
9	—	2	32	32	...	...	...
10 (infected lung)	—	2	4	4	8	8	4
11	—	—	4	8	4	2	2
12	—	2	8	16	8	8	...
13	—	—	2	...	...	...	...

* Titers expressed as reciprocals of serum dilutions.
... Animal died.

TABLE 2

PRODUCTION OF TUBERCULOUS ANTIBODIES FOLLOWING INJECTION OF LUNG SUSPENSIONS

Serums	Titers to OT-sensitized Sheep Erythrocytes (weeks)													
	0		1		2		3		4		5		6	
	HL*	HA	HL	HA	HL	HA	HL	HA	HL	HA	HL	HA	HL	HA
1–5 (normal lung)	—	—	—	—	—	—	—	—	—	—	—	—	—	—
6	—	—	8	—	32	16	—	4	—	16	...	...	...	...
7	—	—	4	—	32	2	16	4	32	16	...	...	...	...
8	—	—	—	—	32	—	8	—	32	16	64	16	...	...
9 (infected lung)	—	—	4	—	64	8	—	8	...	...	...	...	...	...
10	—	—	8	16	16	32	8	8	8	16	8	32	...	...
11	—	—	—	—	16	32	64	32	8	8	2	16	—	8
12	—	—	—	—	32	32	64	64	64	64	16	32	...	...
13	—	—	—	—	—	4	...	...	...	...	...	...	...	...

* HL refers to hemolysis; HA refers to hemagglutination.
... Animal died.

Three such anti-complementary serums and three normal serums (1.0 ml. of each) were mixed with Falba® and mineral oil adjuvants from which tubercle bacilli were omitted (*see* below). All six serum-adjuvant mixtures were injected intramuscularly into normal rabbits. After three weeks the animals were bled and the serums were examined for anti-lung substances and for tuberculous antibodies by the procedures described above.

The results of testing serum samples before and three weeks after injection are compared in table 3. Although no anti-lung activity was demonstrated in the control serums, small amounts were stimulated by the injections of the anti-complementary serums. The absence of tuberculous antibodies suggests that tubercle bacilli or bacillary products were probably not present in the serums transferred into the normal rabbits and, therefore, were not responsible for the production of the lung-specific antibodies demonstrated by the complement-fixation test.

Factors affecting the antibody response: In further studies on the nature of the antibody response to lung tissue, groups of animals were injected by the intravenous, intramuscular, or subcutaneous routes. All of these methods were shown to be inferior to the intraperitoneal route of injection. Attempts to remove the infective principle from a tuberculous lung by pasteurization resulted in a concomitant denaturation of the antigenic specificity.

In order to provoke a heightened antibody response to lung antigen, 2.0 ml. of normal lung suspension were incorporated into adjuvant containing 1.0 ml. of Falba and 2.0 ml. of mineral oil. Five milligrams of killed tubercle bacilli were blended into this mixture. Each of 2 rabbits was injected intraperitoneally with 10.0 ml. of this antigen.

The serologic response of one of these rabbits as compared with that observed in a rabbit which had received tuberculous lung according to the original schedule of injections may be seen in figure 1. Although the rabbit injected with the adjuvant-antigen mixture received only one-sixth of the actual quantity of antigen, as did the animal which had received a total of 25.0 ml. of infected lung (without benefit of adjuvant), the antibody responses were equivalent. However, the time required to attain these titers differed. Maximal titers following injection of normal lung in adjuvant (containing killed tubercle bacilli) were ob-

TABLE 3

LUNG ANTIBODIES PRODUCED BY INJECTING ANTI-COMPLEMENTARY SERUMS

Serum	Injected with	Baseline Titers		Third-Week Titers	
		Anti-Lung	Anti-OT-Sensitized Erythrocytes	Anti-Lung	Anti-OT-Sensitized Erythrocytes
451	Normal serum	—	—	—	—
452	Normal serum	—	—	—	—
453	Normal serum	—	—	—	—
454	Anti-C′* serum I	—	—	4	—
455	Anti-C′ serum II	—	—	8	—
456	Anti-C′ serum III	—	—	4	—

* Anti-C′ refers to anti-complementary serum.

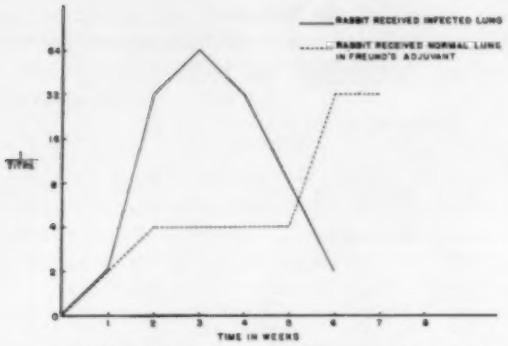

FIG. 1. Response of rabbits to injections of tuberculous lung and to normal lung in Freund's adjuvant.

served after six weeks, while the maximal serum titers of the animal which had received infected lung alone occurred in only three weeks. Infected lung introduced in adjuvant, free of tubercle bacilli, resulted in titers comparable with those produced by the normal lung-adjuvant mixture.

Several attempts were made to ascertain the factor in tuberculous lung tissue responsible for the antibody response exhibited by animals which had received this antigen.

Three rabbits were injected with a mixture of OT and normal lung in a Falba and mineral oil adjuvant, while 2 other rabbits, serving as controls, were injected with OT in adjuvant and normal lung in adjuvant, respectively. Two additional rabbits received the normal lung in Freund's adjuvant, i.e., adjuvant containing tubercle bacilli. Each of the antigen preparations was introduced by intramuscular injection in 5.0 ml. quantities. Serums from weekly bleedings were examined as before.

The results (table 4) indicated that the antibody-stimulating factor was associated with the whole tubercle bacilli (serums 460 and 461) rather than with OT (serums 821, 822 and 823). The general response in these rabbits (460–461) was similar to that observed in the serums of rabbits which had received infected lung alone.

Specificities of the antibody responses: To test the specificity of these anti-lung substances, high titered serums were titrated against kidney, liver, and testes antigens. Although the maximal titer of a typical serum was demonstrated with lung antigen (table 5), the same serum reacted with the other tissue antigens as well. An aliquot of this serum was thoroughly adsorbed with acetone-extracted kidney sediment and was re-examined for complement-fixing antibodies. The adsorption of this serum (460-V) removed all of the cross-reacting or common antibodies while leaving some specific anti-lung substances. Similar results have been reported by Pressman and Eisen (8) while testing heterologous lung systems.

Five serums containing both complement-fixing lung antibodies and tuberculous antibodies were adsorbed with dried, nonviable tubercle bacilli (10 mg. per

TABLE 4

INFLUENCE OF OT AND TUBERCLE BACILLI IN THE PRODUCTION OF ANTIBODIES TO NORMAL LUNG SUSPENSIONS

Serum	Immunizing Agent in Adjuvant	Complement-fixation Titers of Lung Antibodies (weeks)						
		0	1	2	3	4	5	6
821	OT + normal lung	—	—	2	2	2	—	...
822	OT + normal lung	—	—	—	—	—	—	—
823	OT + normal lung	—	—	4	—	—	—	—
824	OT	—	—	—	—	—	—	—
825	Normal lung	—	—	—	—	—	—	—
460	Tubercle bacilli + normal lung	—	—	—	4	4	16	64
461	Tubercle bacilli + normal lung	—	—	—	—	2	8	16

TABLE 5

REACTIVITY OF ANTI-LUNG SUBSTANCES TO HETEROLOGOUS TISSUES BEFORE AND AFTER ADSORPTION

Antigen	Complement-fixation Titers of Anti-Lung Substances	
	Before Adsorption	After Adsorption with Kidney Tissue
Lung	256	8
Kidney	32	—
Liver	16	—
Testes	16	—

TABLE 6

INDEPENDENCE OF ANTIBODIES AS DEMONSTRATED BY RECIPROCAL ADSORPTIONS OF ANTI-LUNG SERUM

Serum	Titers								
	Before Adsorptions			Adsorption with Tubercle Bacilli			Adsorption with Lung Sediment		
	Anti-Lung	Anti-OT-sensitive Erythrocytes		Anti-Lung	Anti-OT-sensitive Erythrocytes		Anti-Lung	Anti-OT-sensitive Erythrocytes	
		HL*	HA		HL	HA		HL	HA
A-4	32	—	16	32	—	—	4	—	16
B-4	16	64	16	16	—	—	—	64	16
D-3	32	—	8	16	—	—	—	—	8
G-4	8	32	8	8	—	—	—	32	8
F-3	8	128	32	8	—	—	—	128	32

* HL refers to hemolysis; HA refers to hemagglutination.

ml. for two hours at 37°C.) and re-examined for each type of immune substance. Additional aliquots of these same serums were adsorbed with acetone-extracted lung sediment and re-tested in the same manner as above (table 6).

Adsorption with tubercle bacilli selectively removed only those antibodies

reactive with tuberculin-treated erythrocytes. Conversely, serums adsorbed with the lung sediment retained their reactivity to tuberculin-sensitized erythrocytes, while in only one serum could any lung antibody be demonstrated and then at a minimally detectable level.

Discussion

Attempts preliminary to ascertaining the possible existence of auto-tissue antibodies in tuberculous disease were concerned with determining if antibody to lung tissue could be produced regularly in the same species.

Since serums from rabbits contain naturally occurring antibodies to constituents of rabbit tissue (Kidd and Friedewald antibodies), it was necessary to eliminate these substances from test serums by heat inactivation (65°C., twenty minutes), in order to measure only those antibodies produced in response to the injection of homologous lung tissue.

The failure to obtain high titers of lung antibodies in the circulating blood suggests the possibility that these substances may become localized in the tissues soon after they are produced. Sarre (9), Frick (2), and Cavelti (1) have observed that antibodies to homologous tissues are at times scarcely demonstrated, and have suggested that they may be consumed by the pathologic process. The inability to demonstrate these antibodies in serum, therefore, does not preclude their existence, as just stated. They may, in addition to being fixed in tissue, exist as circulating antigen-antibody complexes or may defy demonstration because of excess antigen in the circulation. The presence of circulating antigen in some form was evidenced by the production of specific antibodies following injection of normal rabbits with anticomplementary serums from immunized animals.

It is apparent that tubercle bacilli, either viable or nonviable, were necessary to the production of homologous lung antibodies. The injection of normal lung as a saline suspension, or incorporated in an adjuvant from which tubercle bacilli had been omitted, failed to elicit the production of demonstrable antibodies. The addition of killed tubercle bacilli to the adjuvant sufficiently enhanced the antigenic stimulus so that measureable amounts of antibody resulted. A similar pattern of antibody production was effected by injecting infected lung in adjuvant free of bacilli. The earliest antibody response occurred in animals which had received a total of 25.0 ml. of infected lung suspended in saline, but it will be recalled that this quantity of inoculum was six times that present in the antigens introduced in adjuvant.

Although tuberculous infection per se is not required for the production of lung antibodies, no appreciable titer of these substances was observed following injection of normal lung and OT. Hence, it is likely that antibody production is related to the tissue response initiated by tubercle bacilli. This is in keeping with the generally accepted concept of the effectiveness of tubercle bacilli, as in Freund's adjuvant, for enhancing the host response to a variety of parenterally introduced antigens.

It is of interest that attempts to produce serum antibodies by injecting normal lung into previously diseased animals have been unsuccessful to date. Again, it

is possible that, should such antibodies have been produced, they might have localized in infected lung tissue and thus escaped detection.

The serums which contained anti-lung substances also were shown to possess antibodies reactive with other rabbit tissues. However, these heterologous antibodies were adsorbable with acetone-extracted kidney sediment, which reflects the ubiquity of certain tissue components (8), leaving only specific lung antibodies.

Reciprocal adsorptions of test serums with dried mycobacteria and lung tissue emphasized the independent specificities of the corresponding antibodies.

From the data recounted here, it is obvious that efforts to assign auto-tissue antibodies to a possible role in tuberculous disease will require investigative procedures additional to those employed in this study. Several approaches to this general problem are now under consideration.

Summary

Lung-specific antibodies demonstrated by complement fixation were produced in rabbits by intraperitoneal injections of homologous tuberculous lung suspensions or by normal lung with killed tubercle bacilli as an adjuvant. Injection of normal lung alone did not stimulate the production of these substances.

Differences in rate and extent of antibody production were related to the quantity of antigen introduced, the route of injection, and the use of an adjuvant. It would appear that the presence of tubercle bacilli, either living or dead, is necessary for the production of these substances.

Although the test serums contained antibodies reactive with other organ antigens, adsorption with an acetone-extracted kidney sediment completely removed all common antibodies, leaving those singularly specific for the lung. Adsorption with dried tubercle bacilli removed only tuberculous antibodies, whereas serums adsorbed with lung sediment retained their tuberculous reactivity.

Sumario

Producción de Substancias Antipulmonares en los Conejos con Antígenos de Tejido Tuberculoso Homólogo

Se produjeron en los conejos anticuerpos específicos para el pulmón y descubiertos por la fijación del complemento por medio de inyecciones intraperitoneales de suspensiones de pulmón tuberculoso homólogo o de pulmón normal con bacilos tuberculosos muertos como coadyuvantes. La inyección de pulmón normal solo no excitó la producción de dichas substancias.

Las diferencias en la velocidad y la magnitud de la producción de anticuerpos se relacionaron con la cantidad de antígeno introducida, la vía de inyección y el uso de coadyuvante. Parece que, para la producción de estas substancias, se necesita la presencia de bacilos tuberculosos, ya vivos o muertos.

Aunque los sueros ensayados contenían anticuerpos que reaccionaban con antígenos de otros órganos, la adsorción con un sedimento renal extraído con acetona eliminó todos los anticuerpos comunes, dejando solamente los específicos singularmente para el pulmón. La adsorción con bacilos tuberculosos desecados no eliminó más que anticuerpos tuberculosos, mientras que los sueros adsorbidos con sedimento pulmonar retuvieron su reactividad tuberculosa.

Resume

Production de substances anti-poumon chez le lapin par les antigénes homologues de tissus tuberculeux

Des anticorps spécifiques anti-poumons, démontrés par fixation du complément, ont été produits chez les lapins par l'injection intrapéritonéale de suspensions de poumon tuberculeux homologue ou de poumon normal et de bacilles tuberculeux tués comme adjuvants. L'injection de poumon normal seul ne stimulait pas la production de ces substances. Les différences dans le taux et l'étendue de la production d'anticorps étaient en relation avec la quantité d'antigénes introduite, la voie d'injection et l'emploi d'un adjuvant. Il semblerait que la présence de bacilles tuberculeux, vivants ou morts, soit nécessaire à la production de ces substances.

Bien que les sérums des tests aient renfermé des anticorps réagissant avec d'autres antigènes d'organes, l'adsorption, au moyen d'un sédiment de tissu rénal extrait par l'acétone, enlevait complétement tous les anticorps ordinaires, en ne laissant que ceux qui étaient exclusivement spécifiques pour le poumon. L'adsorption avec des bacilles tuberculeux désséchés n'enlevait que les anticorps tuberculeux, alors que les sérums adsorbés par le sédiment de poumon retenaient leur pouvoir de réactivité tuberculeux.

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Case Reports

DIFFUSE MALIGNANT MESOTHELIOMA OF THE PLEURA[1]

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INTRODUCTION

Malignant mesotheliomas are now regarded as a definite entity, and cases are being reported with increasing frequency. They show a wide range of variation, both from the clinical and pathologic standpoints. The histologic differences are attributed to the dual potentialities of the mesothelium which may form either epithelial or mesenchymal elements, as has been demonstrated in tissue culture studies by Stout and Murray (1). Cases of malignant pleural mesothelioma have been reported by Klemperer and Rabin (2), Postoloff (3), Clagett and associates (4), Godwin (5), and recently by Ozzello and Speer (6). Other cases have undoubtedly been described under various classifications, including endothelioma, carcinoma, epithelioma, and sarcoma. Excellent reviews of the literature are included in articles by Hochberg (7) and Godwin (5). The latter reported fourteen cases of malignant mesothelioma, ten of which arose from the pleura and four from the peritoneum. Dawe and associates (8), Dick (9), and McDonald (10) have described similar primary pericardial tumors.

In the case reported below, pleural biopsy disclosed malignancy of unknown origin, and necropsy showed diffuse malignant mesothelioma originating from the left pleura.

CASE REPORT

A 41-year-old white retired naval officer entered the Portland Veterans Administration Hospital on February 9, 1955, with the chief complaint of left chest pain of two weeks' duration. In July and September, 1954, the patient had had attacks of left chest pain with severe cough productive of small amounts of whitish sputum which lasted for about two weeks; he recovered completely. In December, 1954, he noted a definite "lessening of energy." At the time of admission he complained of constant dull left chest pain, occasional unproductive cough, mild febrile sensations in the mid-afternoon, night sweats, and some dyspnea. His weight decreased from 147 to 135 pounds within the two-week period prior to admission.

Physical examination revealed a well-developed and well-nourished male in apparent good health. The significant findings were confined to the chest and included splinting of the left side, dullness, absence of breath sounds over the left base, and a pleural friction rub over the left midlung field.

Routine laboratory studies, including urinalysis, complete blood counts, and various serologic and chemical studies, were normal. A roentgenogram of the chest showed a left pleural effusion obliterating the left diaphragm and a portion of the cardiac silhouette. The lung fields were clear. A left thoracentesis, performed on February 11, 1955, yielded 600 ml. of amber fluid. The embedded sediment revealed mesothelial cells and leukocytes. Thoracentesis was again attempted on February 15, without success. Bronchoscopy was normal. Between February 23 and November 26, the patient ran a Pel-Ebstein type of fever with elevations up to 104°F. Blood counts taken during the febrile periods showed leukocytes up to 28,600 per cu. mm. with a shift to the left. Antituberculous chemotherapy,

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FIG. 1. Roentgenogram of chest, obtained December 27, 1955, showing marked pleural thickening bilaterally.

broad-spectrum antimicrobials, penicillin, and sulfadiazine were of no benefit. A left scalene node biopsy on April 22 showed no abnormality. Left pleural biopsies on May 4 and July 15 were interpreted as metastatic adenocarcinoma and undifferentiated carcinoma, respectively. Following the first pleural biopsy, palliative radiotherapy was given without beneficial results. In June the patient developed anorexia and diarrhea, followed later by hiccups. A gastrointestinal series and barium enema were normal at this time, but in October they showed changes consistent with external pressure on the intestinal tract, with no intrinsic lesions. The blood count on October 21 showed 28,600 leukocytes per cu. mm. with 82 per cent neutrophils, 14 per cent stabs, and 4 per cent monocytes. A chest roentgenogram on December 27 revealed markedly thickened pleura bilaterally, especially on the left side (figure 1). The patient's course in the hospital was continually downhill. Peculiarly, his temperature remained normal for seven weeks prior to death. He died on January 19, 1956.

Autopsy Findings

Macroscopic: The body was that of a cachetic man with numerous small, hard, fixed nodules in the left thoracotomy scar. The left pleural space was completely obliterated by a diffuse white homogeneous mass measuring up to 4.5 cm. in thickness (figure 2). This formed a sheathlike covering of the lung. The cut surface showed this pleural tumor to extend minimally into the lung parenchyma (figure 3). The left lung weighed 1,260 gm. and the right lung, 750 gm. The right pleura was also replaced in part by the same type of thick white tissue as covered the left lung, but to a much less degree. No abnormality was found within the entire bronchial tree. The parabronchial and mediastinal lymph nodes were enlarged and replaced by the tumor. The diaphragm was extensively involved.

The abdominal cavity revealed a diffuse white thickening of the entire peritoneum (figure 2). The liver and splenic capsules were diffusely coated by sheets of tumor which continued over the leaves of the diaphragm and over the entire gastrointestinal tract. The mesenteric, portal, and retroperitoneal lymph nodes showed extensive metastases. The periadrenal tissues were also infiltrated. The kidneys were not involved.

Microscopic examination: The tumor was confined principally to the mesothelial surfaces and the lymph nodes. The peripheral portions of the lungs showed moderate tumor

FIG. 2. Diffuse mesothelioma of pleura and peritoneum.

FIG. 3. Sectioned surface of left lung with mesothelioma.

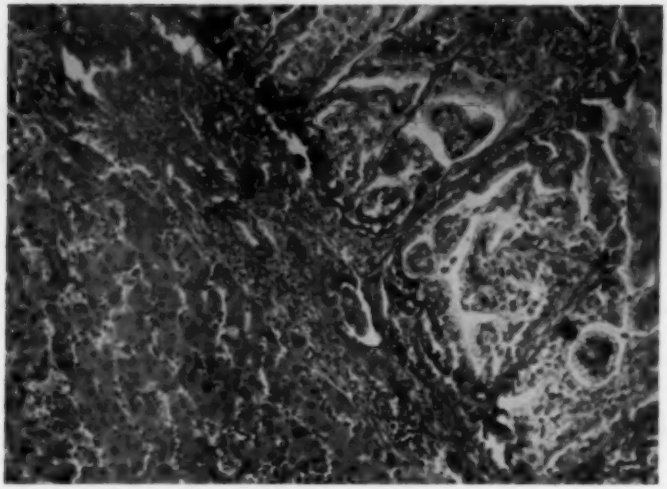

FIG. 4. Microsection of liver with malignant mesothelioma covering the surface and invading the capsule.

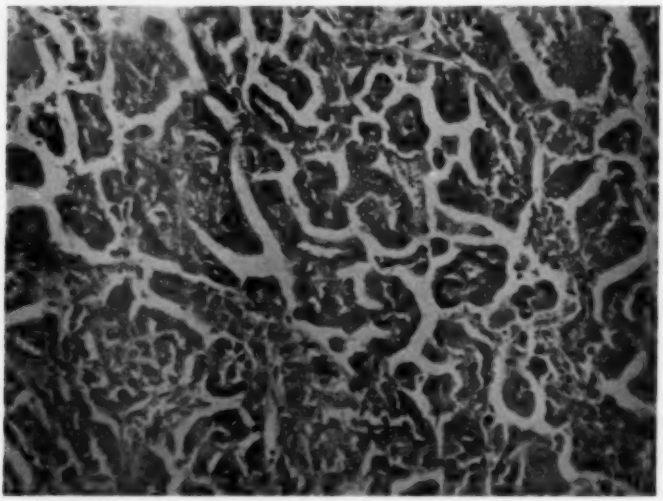

FIG. 5. Microsection of tumor showing papillary pseudoglandular pattern.

involvement by direct extension from the pleura with occasional lymphatic permeation. The liver and spleen were free of parenchymal involvement, but the liver showed areas of capsular invasion (figure 4). There was a tumor in the periadrenal fat with some infiltration of the adrenal glands themselves. The gastrointestinal tract revealed a thick layer of

neoplasm surrounding the serosal surfaces with varying degrees of penetration into the smooth muscle layers. Nowhere did the process extend to the mucosa.

The histologic appearance of the tumor was similar in all of the organs involved. It consisted of compactly arranged epithelial-like cells of moderate size and shape, containing dark-staining rounded or oval nuclei and exhibiting moderate mitotic activity. Pleomorphism was not pronounced. There was a tendency to produce pseudoglandular structures having a distinct papillary pattern (figure 5). The cells contained moderate amounts of cytoplasm without evidence of secretory activity. This was confirmed by a PAS stain from the pleural biopsy specimen. In some areas the stroma contained dense bands of hyalinized connective tissue.

Comment

This case presents several interesting clinical features, some of which have not been previously described. The most unusual one was the Pel-Ebstein type of fever curve which was present for about eight months. The patient was afebrile, however, during the last seven weeks of his illness. The fact that the left pleural effusion disappeared following one thoracentesis seemed unusual. The finding of an amber pleural fluid rather than a bloody one was misleading. The complete absence of lymphocytes in the peripheral blood three months prior to death remains unexplained.

The initial clinical impression was that of a tuberculous or pneumonic effusion. Because of the lack of response to specific chemotherapy and the appearance of the Pel-Ebstein fever, other etiologies were considered, including Hodgkin's disease. Until the time of pleural biopsy the exact nature of the disease process was not ascertained. Complete necropsy alone disclosed the origin of the neoplasm.

The main pathologic feature of interest was the manner of spread of the tumor, namely, over mesothelial surfaces producing widespread thickenings of the serous membranes. This has been described by the German writers as "Geschwulstpanzer." There was no significant visceral parenchymal involvement. The appearance of the left pleura and the absence of any other primary site for the tumor left no doubt of its origin. The capsule of the liver and spleen seemed resistant to parenchymal spread. The writers believe that the manner of spread from the left pleura was by direct extension and lymphatic permeation to the diaphragm and thence to the right pleura and peritoneal cavity. No blood-borne metastases were observed.

Pleural biopsy is of great aid in establishing the diagnosis of malignancy but is often inconclusive as to the type of neoplasm. Radiotherapy was of no avail in the present case.

The existence of a malignant tumor arising from serous membranes has been questioned by some observers. They are of the opinion that it represents a metastatic lesion from some undiscovered primary source, the most frequent site of origin being the lungs. Godwin (5) has examined large numbers of proved bronchogenic carcinomas, and none of these "duplicated the gross patterns and metastatic behavior of mesotheliomas." This has also been the experience of the present writers in a series that they have studied. Ozzello and Speer (6) believe that the demonstration of mesothelial outgrowth in tissue cultures is convincing evidence of their histogenesis from mesothelial cells.

The present case fulfills the criteria for a primary malignant mesothelioma. There was no other recognizable primary source, and the pathologic findings were almost identical with previously reported cases. The absence of mucin within the tumor cells, as demonstrated by the PAS and mucicarmine stains of post-mortem material, strengthens the writers' interpretation. The gross photographs and microscopic slides were reviewed by Drs. Warren C. Hunter, Ernest J. Losli, and Abe Oyamada, who consider this case as one

of primary mesothelioma. Other reviewers were somewhat uncertain of this entity and were consequently reluctant to give a definite opinion.

Summary

A case of primary malignant pleural mesothelioma is reported. Clinical findings included a Pel-Ebstein type of fever, a rapid disappearance of an amber pleural fluid, and a complete absence of lymphocytes in the peripheral blood during the later stages of the disease. Histologically, the tumor resembled a carcinoma and was so diagnosed by two pleural biopsies. The exact type of malignancy was disclosed at necropsy.

Sumario

Mesotelioma Maligno Difuso de la Pleura

Preséntase un caso de mesotelioma maligno primario de la pleura. Los hallazgos clínicos comprendieron fiebre tipo de Pel-Ebstein, rápida desaparición de un líquido pleural ambarino y falta total de linfocitos en la sangre periférica durante los últimos períodos de la dolencia. Histológicamente, el tumor parecía carcinoma y así fué diagnosticado por dos biopsias pleurales. En la autopsia se descubrió la forma exacta del cáncer.

Resume

Mésothéliome malin de la plèvre

Un cas de mésothéliome malin primaire de la plèvre est rapporté. Les signes cliniques consistaient en une pyréxie du type Pel-Ebstein, une disparition rapide d'un liquide pleural ambré et une absence complète de lymphocytes dans le sang circulant au cours des derniers stades de l'affection. Histologiquement, la tumeur ressemblait à un carcinome, ce qui fut le diagnostic porté à la suite de deux biopsies pleurales. Le type de cette tumeur n'a été découvert qu'à l'autopsie.

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CAPLAN'S SYNDROME[1]

A Perplexing Pneumoconiosis with Rheumatoid Arthritis

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INTRODUCTION

That pulmonary changes develop during the course of the various types of so-called collagen diseases is a well-recognized clinical fact, and it is therefore not surprising that such lesions have been described in rheumatoid arthritis. These "rheumatoid" pulmonary lesions, in the absence of pneumoconiosis, do not present a characteristic roentgenographic appearance but, in the presence of such a dust disease, they may do so.

In 1953 Caplan (1), a member of the Cardiff Pneumoconiosis Medical Panel, first drew attention to the association between rheumatoid arthritis and unusual chest roentgenographic changes which he encountered among coal miners of South Wales. These changes were quite distinct from any of the roentgenographic types of anthracosilicosis ordinarily encountered, including the stage of conglomeration (stage III, or progressive massive fibrosis). They were characterized by the presence of multiple, well-defined, round opacities varying in diameter from 0.5 cm. to 5.0 cm., scattered throughout both lungs on a background in which other evidence of pneumoconiosis was usually minimal. These opacities had a tendency to appear in "crops" and to develop fairly rapidly in contrast to the slow formation of the ordinary anthracosilicotic conglomerate nodules. They might appear before, coincident with, or following the rheumatoid arthritis. Often remaining stationary for years, these lesions either continued unchanged, calcified, or contracted to leave a small scar; they might break down into cavities, which on occasion went on to complete closure without residuals (2). It should be emphasized that these changes were noted only in men exposed to a dust hazard and have no similarity to the interstitial pneumonitis of rheumatoid arthritis in patients without silicosis described in 1948 by Ellman and Ball (3).

To determine the validity of this new syndrome, an epidemiologic survey among coal miners was conducted by Miall and associates (4). From among 896 chest films of coal miners showing either progressive massive fibrosis or significant tuberculosis, 20 films were selected as demonstrating the characteristic opacities of rheumatoid arthritis. A group of fifty-nine control subjects were chosen on the basis of films that were rejected because they did not show these lesions. A physician unaware of the roentgenographic diagnoses then examined all members of both groups. Among these fifty-nine control subjects there were two (3 per cent) who had rheumatoid arthritis. Examination by the same physician of additional control groups that included ninety-two examples of simple pneumoconiosis and one hundred and twelve persons with normal chest films failed to uncover any cases of rheumatoid arthritis. In contrast, among the 20 patients with the Caplan type of pulmonary lesions, eleven (55 per cent) were found to be suffering with rheumatoid arthritis, while nine (45 per cent) showed no evidence of this disease.

In 1955, Gough and co-workers (5) published their description of the pathology of the lung lesions in sixteen coal miners with rheumatoid arthritis. In all but two, these were post-mortem studies. Grossly, the nodules resembled the round opacities seen

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roentgenographically. Macroscopically, on section, they showed a concentric arrangement of lighter and darker layers, the lighter often representing areas of liquefaction. Some nodules were calcified. The intervening lung tissue showed only slight or moderate pneumoconiosis. Microscopically, the nodules consisted of necrotic centers containing collagen, surrounded by free-lying dust often arranged in circles, outside of which was a concentric layer of collagen. In about one-half of the cases, tuberculosis was recognizable histologically.

Since Caplan's original description in 1953 (1) and the subsequent report by Miall and associates (4), additional cases have been reported from the European continent. Clerens (2) described examples in two Belgian female factory workers exposed to a silica hazard; Christiaens and co-workers (2), in six French coal workers; and Balgaires (2), in thirty French coal workers, all of whom exhibited this syndrome. In addition, Petry (2) found two such cases among German coal miners, and Martin and Fallet (2) reported one from Switzerland.

As far as is known, the American literature to date has contained no case report of Caplan's syndrome. With this hospital located in the anthracite coal mining area, an opportunity occurred to study a patient with this disease, and his case is the basis of the present report.

Case Report

A 64-year-old white male was admitted to this hospital on August 6, 1957, with a diagnosis of anthracosilicosis; he had been a hard-coal miner for approximately twenty years. The patient's complaints were chiefly referable to the pulmonary and cardiovascular systems. In 1950 the appearance of dyspnea on limited exertion forced him to retire from the mines. With the passage of time, this symptom slowly became manifest on less and less exertion. About seven months prior to admission, this course of events was complicated by the appearance of dyspnea at rest and swelling of the feet. Treated at home with rest, digitalis, and mercurial diuretics, the patient experienced a gradual recovery which was complete in several weeks. Subsequently, on maintenance digitalis therapy his symptoms did not recur. However, exertional dyspnea, such as that produced by going up one flight of stairs, had persisted.

The patient gave no history of rheumatic fever, hypertension, coronary insufficiency, or paroxysmal nocturnal dyspnea.

In addition to the exertional dyspnea, during the winter seasons of the past few years, he had suffered with "chest colds" characterized by rather marked cough and black expectoration. At the time of hospitalization, however, both of these symptoms were only minimal. Hemoptysis had never been observed.

Furthermore, the patient had arthritic, genitourinary, and gastrointestinal complaints. In 1950 he experienced (only during the winter) swelling and pain limited to the finger joints of both hands. In 1951, during the same season, there was a recurrence of the arthritic symptoms. Subsequently he had no trouble with these or any other joints. With regard to the genitourinary system, his only complaint, which had been present for several years, had been that of awakening to urinate once or twice nightly. His gastrointestinal disturbance, also present for several years, had been a transient sensation of abdominal fullness after meals. There was no history of a recent loss of weight.

Physical examination on admission revealed a 64-year-old-white male, fairly well developed and nourished, appearing quite well. He showed no dyspnea at rest but, on donning his hospital clothes after the examination, he became somewhat short of breath. His temperature was 98.4°F., the blood pressure was 170 systolic, 110 diastolic in mm. of mercury; and the pulse rate, 76 per minute, with the pulse regular in force and rhythm. The chest was symmetrical, its expansion being limited bilaterally. The lungs disclosed a few scattered expiratory wheezes. The heart was not enlarged nor were there any thrills. However,

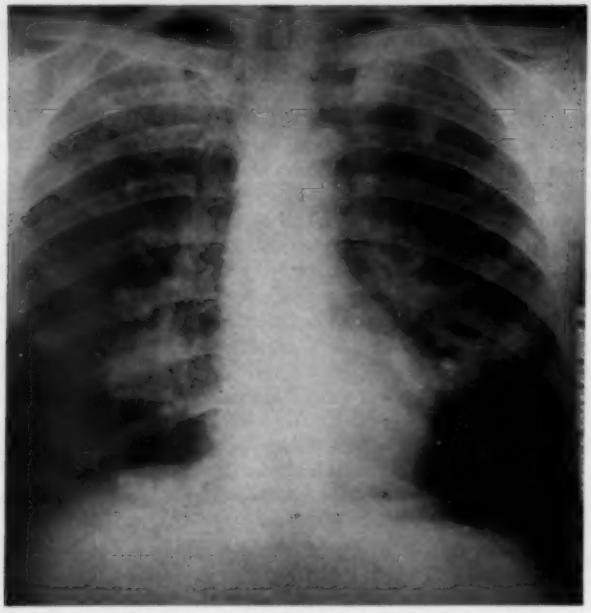

FIG. 1. Roentgenogram (May 10, 1948), showing the conglomerate lesions of third-stage anthracosilicosis or progressive massive fibrosis. The heart is normal in size.

FIG. 2. Roentgenogram (August 7, 1957) showing the numerous bilateral round opacities of the Caplan type. Left ventricular hypertrophy is noted.

a grade III systolic murmur was audible at the mitral area; a grade II systolic murmur, at the aortic area, with the second sound markedly diminished in intensity over the aortic area. The rest of the examination, including that of the joints, was found to be normal.

Laboratory data: The following examinations were normal: complete blood count, blood sugar, urea nitrogen, uric acid, acid phosphatase, and serum albumin and globulin. Examination of two peripheral blood preparations for lupus erythematosus cells was negative. The serologic (V.D.R.L.) test for syphilis was negative. A skin test with tuberculin purified protein derivative, first strength, was negative, but the second-strength test with the same material yielded a 3-plus reaction. Examination of several fresh sputum specimens and of several similar specimens, each collected for twenty-four hours, revealed neither fungi nor acid-fast bacilli on smear and culture. An electrocardiogram disclosed T wave and ST segment changes indicative of digitalis effect or left ventricular hypertrophy, or both. Ventilatory studies showed evidence of moderate impairment of pulmonary ventilation, obstructive in type: the vital capacity was 2.5 liters or 72 per cent of the predicted value; the maximal breathing capacity, 48.0 liters per minute or 53 per cent of the predicted value; and the air velocity index, 0.74.

Roentgenographic data: An intravenous pyelogram, a gastrointestinal series, and a barium enema were all normal. The roentgenograms of both hands were suggestive of either gout or rheumatoid arthritis. Oblique chest films were taken, with barium in the esophagus: the right oblique film was normal, but the left showed enlargement of the left ventricle. Fortunately it was possible to obtain two previous chest films, dated May 10, 1948, and July 18, 1950. The roentgenogram of the chest taken in 1948 (figure 1) showed the conglomerate lesions of third-stage anthracosilicosis or progressive massive fibrosis; the heart was normal. The 1950 film was essentially similar. The hospital admission roentgenogram of the chest, obtained on August 7, 1957 (figure 2) showed numerous round opacities on both sides varying between 1.5 and 8.5 cm. Two of the opacities on the right were larger than 5.0 cm., one being 5.5 cm. and the other 8.5 cm. In addition this film showed evidence of left ventricular hypertrophy. Fluoroscopic examination of the chest revealed not only the pulmonary opacities and enlarged left ventricle seen in the roentgenograms, but also small pulsating calcific deposits in the region of the aortic valve.

Endoscopic data: Examination by means of the bronchoscope and the sigmoidoscope revealed no abnormalities.

Biopsy report: A scalene node biopsy on the right was performed on August 19, 1957. Histologic examination of 3 small lymph nodes, which were removed, showed only evidence of reactive hyperplasia. There was no evidence of silicosis, tuberculosis, or malignant disease.

Course in hospital: Because of the history of cardiac failure, and the presence of cardiac abnormalities noted during his hospitalization, the patient was treated with a 600-mg. sodium diet and continued on maintenance doses of a digitalis preparation. For the chronic bronchitis, he received bronchodilators. Blood pressures repeatedly taken showed systolic pressures ranging between 150 and 180 and diastolic pressures between 100 and 110. As his physical activity was limited, the patient became practically asymptomatic, and on August 30, 1957, he was discharged from the hospital. He was recalled for follow-up examination on January 27, 1958. At that time, he still complained of exertional dyspnea and he still appeared quite well. A chest roentgenogram obtained on that date was essentially similar to that obtained during his hospitalization in 1957.

Comment: The results of the investigation led to the following diagnoses: pneumoconiosis of the Caplan type associated with cured rheumatoid arthritis of the hands, the so-called Caplan syndrome; chronic bronchitis with pulmonary emphysema; essential hypertension; and heart disease due to hypertension and possibly rheumatoid arthritis, with calcific aortic stenosis and left ventricular hypertrophy but without cardiac failure.

The first impression, when the 1957 chest film was seen, was that multiple metastatic

malignant lesions were present in both lungs. At that time, the 1948 and 1950 roentgenograms were not available. However, the patient's physical well-being, benign course in the hospital, and the results of the various examinations made it appear that malignant disease was not responsible for the roentgenographic changes. It was believed that the first signs of the pneumoconiosis of the Caplan type were visible in the 1948 chest film, not as typically round opacities but as conglomerate lesions of third-stage anthracosilicosis. The 1957 chest film, however, displayed typical Caplan lesions, many of which had developed from the conglomerate masses. It should be noted that two of the opacities were larger than 5.0 cm., a measurement given in the literature as the upper limit for such lesions. From bacteriologic and histologic standpoints there was no proof that these lesions were tuberculous.

With the roentgenograms of the hands suggestive of either gout or rheumatoid arthritis, but without clinical or laboratory evidence of gout, it was thought that the patient had recovered from rheumatoid arthritis, the clinical manifestations of which were evident in 1950 and 1951. It should be recalled that conglomerate disease was already present in the chest film of 1948, prior to the development of the arthritic symptoms.

* * *

In the experience of the present writers, chronic bronchitis and pulmonary emphysema are not infrequent complications of conglomerate anthracosilicosis. Since the Caplan type of pneumoconiosis may be regarded as a form of conglomerate anthracosilicosis, it is not surprising that the patient had both of these complications. They account for the exertional dyspnea of which he complained.

The hypertension in this patient was thought to be merely coincidental. On the other hand, the finding of calcific aortic stenosis, which has been noted to be associated with rheumatoid arthritis (7), is possibly more than a mere coincidence. That this may be so was indicated by a review of 183 other cases of rheumatoid arthritis treated at this hospital which will be described in more detail later. Among these cases, there were 4 patients with calcific aortic stenosis, an incidence of 2.2 per cent. None of these patients had anthracosilicosis and, to the writers' knowledge, this type of valvular disease has not been previously reported in the literature in patients showing this peculiar form of pneumoconiosis with or without rheumatoid arthritis.

Incidence of Caplan's Syndrome at This Hospital

Stimulated by the fortuitous finding of the one case just reported, it was decided to investigate the frequency of the Caplan type of pulmonary lesions in this hospital. The chest roentgenograms of 100 coal miners consecutively discharged with a diagnosis of anthracosilicosis, but without rheumatoid arthritis, were reviewed. In addition, because of the suggested association of tuberculosis with anthracosilicosis in the production of the rheumatoid lung changes, the chest films of 35 patients, none of whom had rheumatoid arthritis but all of whom had active silicotuberculosis, were also examined. None of this combined group of 135 patients showed the pulmonary lesions described by Caplan.

Furthermore, although it was recognized that the Caplan type of pulmonary lesions has not been described in rheumatoid arthritis without silicosis, the clinical records and roentgenograms of all patients discharged from this hospital with a diagnosis of rheumatoid arthritis during the period extending from November, 1950, to June, 1957, were evaluated. These patients, which included 4 with aortic stenosis, have been alluded to previously. Excluding the case described, the group was found to consist of 183 persons who had a total of 306 hospital admissions. A chest roentgenogram had been obtained

at the time of each admission, and additional films were taken when it appeared to be clinically indicated. This group was found to include 15 patients with anthracosilicosis. In one of these, the anthracosilicosis was classified as stage I (increased pulmonary markings); in 6, it was classified as stage II (with scattered nodularity); and, in 8, it was classified as stage III (with conglomeration). In one of the latter cases, two large conglomerate masses, one on each side, were found to have broken down into cavities, but on post-mortem examination no evidence of tuberculosis was found. In none of the 15 with anthracosilicosis did the chest films show the Caplan type of lesion.

In addition, among the patients with rheumatoid arthritis without evidence of anthracosilicosis, there were 7 who suffered with other pulmonary diseases. These included minimal active pulmonary tuberculosis in one, bronchopneumonia in 3, and single instances of lobar pneumonia, pleurisy of undetermined etiology, and bronchial asthma. In these patients, there was nothing in either the clinical course or roentgenographic changes which would differentiate their disease from similar lesions occurring in patients without rheumatoid arthritis. None of the pneumonias could be accepted as unequivocal examples of the acute rheumatoid pneumonitis as described by Ellman and Ball (3) and others (8–10).

Discussion

The exact etiology of the pulmonary changes in anthracosilicosis with rheumatoid arthritis remains in the field of speculation (2). The possibility that the lung lesions are purely a manifestation of the rheumatoid diathesis and are unrelated to pneumoconiosis has not found acceptance. A deficiency of adrenocortical hormone was tentatively proposed by Miall and associates (4) to account for both the joint and pulmonary disturbances, but such a deficiency cannot be satisfactorily demonstrated. Kellgren (11) suggested that rheumatoid arthritis was accompanied by a breakdown of collagen, and that these changes in the lung would be made more radio-opaque by the migration of dust-laden phagocytes to the areas of inflammation. Miall accepted this explanation as possible but highly improbable. The hypothesis receiving the most serious consideration at present is one which includes *M. tuberculosis* as one of the etiologic factors. There are both post-mortem and some clinical studies which tend to incriminate tuberculosis in the production of lung lesions (6). It has been suggested that, the collagen tissue response having been altered by the rheumatoid state, the tuberculous or modified tuberculous lesions which ordinarily result in progressive massive fibrosis in patients with pneumoconiosis produce instead the circumscribed opacities characteristic of the Caplan syndrome (2). This theory, of course, fails to explain the presence of the lung lesions in the nine (45 per cent) patients in Caplan's original study who had no rheumatoid arthritis. However, this criticism applies equally to every hypothesis which has been presented to date so that it is obvious that for the present the etiology of this syndrome remains purely conjectural.

Despite this, awareness of the syndrome is of practical value. The one condition with which it is most likely to be confused is metastatic malignancy of the lung. Although the possibility of metastatic malignancy should be thoroughly investigated, failure to find a primary tumor in a patient with anthracosilicosis and rheumatoid arthritis still permits a reasonable explanation of the cause of the pulmonary lesions.

Diagnosis of the Caplan syndrome or the Caplan pneumoconiosis alone carries with it attendant therapeutic implications. As in every case of progressive massive fibrosis, a diligent search should be made to find tubercle bacilli in sputum or gastric washings. If the bacilli are present, a course of antituberculous chemotherapy is indicated immediately. If the tubercle bacilli are not found but the tuberculin skin reaction is positive, the pa-

tient should be kept under close observation and should be followed with periodic chest roentgenograms and bacteriologic studies; a rapid increase in the size of the opacities or the sudden appearance of new ones should probably be considered an indication for antituberculous therapy. If cavitation should develop in any of the circumscribed lung lesions, and the sputum should become positive for tubercle bacilli, treatment should be started. Cavitation, with sputum persistently negative for tubercle bacilli, however, is not an indication for chemotherapy. Experience at this hospital with patients with anthracosilicotic cavities has shown that failure to find tubercle bacilli in the sputum usually indicates that the cavitation is due to nontuberculous causes (12). This was demonstrated again by the post-mortem findings of the one patient in the present study who had rheumatoid arthritis and anthracosilicosis with nontuberculous bilateral cavitation.

In brief, a case of Caplan's syndrome has been described which shows several differences from the case originally described by Caplan. First, many of the pulmonary opacities developed from the conglomerate lesions of third-stage anthracosilicosis; second, two of the opacities were larger than 5.0 cm.; third, calcific aortic stenosis which may have more than a coincidental relationship with rheumatoid arthritis was found.

Summary

Multiple, well-defined, round opacities appearing in the lungs of patients with pneumoconiosis have been described by Caplan as being frequently associated with rheumatoid arthritis. This may be defined as Caplan's syndrome. However, the Caplan type of pulmonary lesion may appear in the absence of rheumatoid arthritis.

A case demonstrating this syndrome, apparently the first to be reported in the American literature, has been presented. There was present, in addition, calcific aortic stenosis, which may be etiologically related to the rheumatoid arthritis.

Experience at this hospital indicates that the Caplan type of pneumoconiosis is a rare occurrence. The case reported is the only one found in a review of 150 patients with anthracosilicosis, 15 of whom had associated rheumatoid arthritis and 35 of whom had complicating silicotuberculosis.

Sumario

El Síndrome de Caplan: Neumoconiosis Intrincada con Artritis Reumatoidea

Ciertos focos opacos, redondos, bien definidos y múltiples que aparecen en los pulmones de enfermos de neumoconiosis fueron descritos por Caplan como asociados frecuentemente con artritis reumatoidea. Cabe llamar a este fenómeno el síndrome de Caplan. Sin embargo, puede aparecer la lesión pulmonar de tipo Caplan aun sin haber artritis reumatoidea.

Se presenta aquí un caso que ilustra este síndrome, aparentemente el primero que se haya descrito en la literatura estadunidense. Había además estenosis aórtica calcificada, que puede enlazarse etiológicamente con la artritis reumatoidea.

Lo observado en el hospital del A. indica que la neumoconiosis de forma Caplan es un fenómeno raro. El caso descrito es el único encontrado en un repaso de 150 enfermos que tenían antracosilicosis, 15 de los cuales tenían además artritis reumatoidea y 35 tenían como complicación silicotuberculosis.

Resume

Syndrome de Caplan: Pneumoconiose surprenante associée à la polyarthrite chronique évolutive

Des opacités rondes, multiples, bien délimitées apparaissant dans les poumons des malades atteints de pneumoconiose ont été décrites par Caplan comme étant fréquemment associées à la polyarthrite chronique évolutive.

Les auteurs présentent un cas où ce syndrome est évident, c'est probablement le premier ayant été publié dans la littérature américaine. Il existait en outre une sténose aortique avec calcification, dont la relation étiologique avec la polyarthrite chronique évolutive était possible.

L'expérience acquise dans cet hôpital indique que la pneumoconiose du type Caplan est rare. Le cas rapporté est le seul que ait été découvert dans l'analyse de 150 cas d'anthraco silicose, dont 15 étaient associés à la polyarthrite chronique évolutive et 35 avec des complications silico-tuberculeuses.

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Books

ANATOMIE MEDICO-CHIRURGICALE DU POUMON. By Michel Latarjet and Felix Magnin. Pp. 326 with 155 figures, G. Doin & Cie., Paris VI°, France, 1956. Price, Fr. 8.500.

This outstanding book is written for students and doctors interested in anything pertaining to the lung. The authors would like it to be a practical and often-used working instrument with which familiarity is rapidly attained.

This book fulfills its authors' expectations because of clarity of exposition, simplicity of style, originality of approach, and the high quality of its intellectual content. The text is readily understandable by anyone having had two years of conversational French. The authors admit the conciseness of the text and cite the work of others for further details in anatomy, histology, and clinical correlation between structure and function. Significant references are listed at the bottom of each page. The different research methods used to obtain the textural material are described.

One of the oustanding features of the book is its actual format. It measures 7½ by 10¼ by 1½ inches and consists of a stout buckram looseleaf notebook covered with water-repellant material. The type is crisp and widely spaced on an excellent quality of paper. The excellent illustrations are colored, line, or half-tone drawings closely related to the text. A three-dimensional effect is present throughout. Bronchoscopy and dissection are illustrated. "Exploded" diagrams help to clarify the course of bronchi and blood vessels in the different pulmonary segments. At the end of the text are plates illustrating bronchograms and relationships of intrathoracic viscera, bronchial and vascular structures (lobar and segmental), and lymphatic drainage. These can be released readily from the looseleaf folder and placed in their desired location in the text by the reader. The excellent table of contents is brief and well organized.

The book is divided into six sections whose details are developed in a precise logical fashion. The first two sections, in a discussion of general characteristics and morphology, relate general physical properties to pathologic changes and include a detailed description of the interlobar fissures and their variations. In Part Three the common relationships peculiar to each lung are described. Cross-section anatomic diagrams are advantageously used in this section. The apical and thoraco-abdominal relationships are emphasized by sagittal sectional drawings. Part Four includes a particularly detailed study of the various hilar structures and their relationship to the pericardial pleura and to one another. Numerous illustrations clarify the course of nerves, lymph drainage, and bronchial vessels.

Part Five describes the lobes of the lungs individually and each segment thereof. Each lobe is discussed from the point of view of its shape and topography. The pedicle is then considered and each segment of the lobe is studied in terms of the distribution of the segmental bronchi and venous drainage system. Detailed drawings illustrate the more frequent anomalous variations of arteries and segmental bronchi.

The sixth portion is entitled "Intrapulmonary Distribution." It is the essence of what has gone before. The distribution of the bronchi, pulmonary artery and veins, bronchial vessels, lymphatic drainage, and nerve supply is carefully developed from the hilum to the periphery.

This clear, concise and beautifully illustrated original approach to the medical and surgical anatomy of the lung should appeal particularly to those interested in a better anatomic correlation between the pathology and treatment of all pulmonary disease.

KEITH MERRILL, JR.
Brookline, Massachusetts

THE POSTOPERATIVE CHEST. By Hiram T. Langston, Anton M. Pantone, and Myron Melamed. Pp. 228, Charles C Thomas, Springfield, Illinois, 1958. Price, $8.00.

The title of this volume is misleading. It is an atlas of many of the commonly observed changes in the roentgenographic appearance of the chest following thoracic surgery. Aside from a brief foreword, the text consists entirely of interpretations of roentgenograms which have been selected to illustrate what the authors choose to term "normal" postoperative findings as well as a number of frank complications.

The authors have attempted to categorize these postoperative changes and to present illustrative roentgenograms in accordance with this tabulation. This has not been entirely successful, as may be seen by the fact that the problem of intrapleural air is covered in several widely separated sections. Reproductions of the roentgenograms approximate, in size, a 70-mm. film, and the quality of the reproductions is excellent. The text of the interpretations is succinct and well-worded and has been assisted in all cases by accompanying line drawings which focus the reader's attention upon the details under discussion. Despite this, one is often painfully aware of the inadequacy of even the best of reproductions in depicting the subtle details which are of such import in reading accurately such roentgenograms. In some instances, the drawings outline changes which the reader cannot, by any stretch of the imagination, discern on the roentgenogram as reproduced.

Although in the foreword the authors have kind words to say about the use of laminagraphy during the postoperative period, they have unfortunately confined their selections to conventional roentgenograms, utilizing only the anteroposterior and lateral projections and including several wherein contrast media has been employed. The teaching value of these roentgenograms would have been further enhanced had the surgeon employed radiopaque catheters for drainage of the chest. Indeed, the authors themselves would probably have been assisted in their own interpretations if the exact location of the drainage tubes had been readily apparent.

Approximately 80 sets of roentgenograms have been selected in such a manner as to provide wide coverage. Despite this, there are several noteworthy omissions, such as massive atelectasis and the use of plastic spheres or sponge for space-filling or collapse. At the same time, it is surprising to find that three of the patients have undergone procedures involving the use of a paraffin pack. There can be little room for disagreement regarding the interpretation of the roentgenograms since the reader is confined to the reproductions, whereas the authors have made their readings from the original films. This reviewer is surprised to find the authors perplexed as to the etiology of several large "chronic apical pneumothoraces" in patients who have undergone resections of a single lobe or less. In these cases, the circumstantial evidence favoring a diagnosis of bronchopleural fistula is well-nigh overwhelming.

This volume is the second in the John Alexander Monograph Series, of which, *The Surgical Management of Pulmonary Tuberculosis* was the first. Due to the difficulties inherent in the material presented, this monograph is a less noteworthy contribution than was the first volume in the series. However, this volume does have considerable merit which is enhanced by the fact that this subject has not been covered so well by any previous publication.

RICHARD F. KEIFFER, JR.
Baltimore, Maryland

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American Trudeau Society

Medical Section of the National Tuberculosis Association

ABSTRACTS OF MEDICAL PAPERS PRESENTED AT THE ANNUAL MEETING OF THE AMERICAN TRUDEAU SOCIETY HELD IN CONJUNCTION WITH THE MEETINGS OF THE NATIONAL TUBERCULOSIS ASSOCIATION AND THE NATIONAL CONFERENCE OF TUBERCULOSIS WORKERS, MAY 18–23, 1958

1A Tuberculosis Including Chemotherapy

Kanamycin in Experimental Guinea Pig Tuberculosis

KEN YANAGISAWA and KOOMI KANAI, Tokyo, Japan

Guinea pig experiments were conducted to test for antituberculous activity of kanamycin, a newly discovered antimicrobial by Umezawa and associates. The animals infected intravenously or subcutaneously with various drug-resistant or -susceptible strains of tubercle bacilli were treated by daily administration of kanamycin (10 or 20 mg. free base per animal), starting one day or two to six weeks after infection. The effect was generally compared with that of the same amount of streptomycin and isoniazid. Evaluation of the efficacy was made by enumeration of viable bacterial cells surviving in the spleen, liver, and lung. In some cases, histologic examination was concomitantly made. Kanamycin activity was found remarkable, exerting the same grade of beneficial effect as streptomycin and isoniazid. No cross resistance was found *in vivo* among kanamycin, streptomycin, isoniazid, and PAS. In addition, no acute and delayed toxicity was observed even when the drug was administered for as long as fifteen weeks (tables 1, 2, and 3).

Next, four strains of kanamycin-resistant tubercle bacilli were isolated in a single step from a large population of bacillary cells of the H2 strain (a virulent human strain) sown on Kirchner agar medium containing kanamycin in 100 γ free base per ml. They were then subcultured thrice on such medium to obtain a "pure" population of drug-resistant cells. A virulence test by guinea pig inoculation indicated that these four strains are pathogenic to guinea pigs in the same way as their parent H2 strain. An attempt was made to examine the therapeutic effect of streptomycin and isoniazid in guinea pigs infected with one of the kanamycin-resistant strains named above. Development of tuberculosis was completely inhibited by those two agents (table 4).

The Antituberculous Activity of Kanamycin in Vitro and in the Experimental Animal (Guinea Pig)

W. STEENKEN, JR., VINCENT MONTALBINE, and J. R. THURSTON, Saranac Lake, N. Y.

Kanamycin inhibits the growth of the human H37Rv strain of tubercle bacilli *in vitro* in a concentration of 1.6 γ per ml. in Tween®-albumin medium. There was growth in the 5 γ per ml. tube but none in the 10 γ per ml. tube when grown in Proskauer and Beck medium plus 10 per cent horse serum. A strain of photochromogenic mycobacteria grew in the presence of 2.5 γ per ml. but was inhibited by a concentration of 5 γ per ml. in Proskauer and Beck medium without serum. Strains of *Mycobacterium tuberculosis* resistant to seromycin, streptovaricin, streptomycin, and isoniazid were all susceptible to 1.6 γ of kanamycin per ml., whereas the neomycin- and viomycin-resistant strains were resistant to 1,000 γ of kanamycin per ml. The drug had definite but limited bactericidal activity. The strain of H37Rv mycobacterium increased in resistance quite rapidly to 1,000 γ per ml. after four transfers in Tween-albumin medium containing the drug. Definite beneficial effect was noted in experimental tuberculosis in guinea pigs infected intracardially and

treated once daily with 30 to 40 mg. of kanamycin per kg. administered intramuscularly. The drug was much less active *in vivo* than isoniazid and slightly less than streptomycin. Kanamycin is far less toxic in the guinea pig than neomycin, even though it showed cross resistance with the latter drug.

Kanamycin: A New Tuberculostatic Agent

KENNETH WRIGHT, ATTILIO RENZETTI, JOSEPH LUNN, PAUL BUNN, DONALD JOHNSON, and OLGA KRAJNJAK, Syracuse, N. Y.

Kanamycin, an antimicrobial derived from *Streptomyces kanamyceticus*, inhibits growth of human tubercle bacilli *in vitro* at concentrations ranging from 0.4 to 5.0 γ per ml. As it proved to be reasonably nontoxic in laboratory animals, a clinical trial of the drug in 12 patients with advanced pulmonary tuberculosis was instituted. Pharmacologic properties, including toxicity, were measured in these patients during five to six months of continuous administration in daily intramuscular doses ranging from 0.25 to 1.0 gm.

Blood concentrations after 1 gm. daily in a single intramuscular dose exceeded 15 γ in all patients at one hour (average concentration, 28 γ per ml.). Concentrations of more than 1 γ persisted for more than twenty-four hours. One hour after a 0.25 gm. dose, blood concentrations averaged 9 γ per ml.; at twelve hours it was 5 γ per ml. There was no absorption after oral doses of 5 gm.

Urinary excretion indicated that the agent was rapidly absorbed and excreted; more than 40 per cent of a single dose was measurable in the urine within twenty-four hours.

Kanamycin exhibited a pattern of toxicity like that of streptomycin. After a 1 gm. single daily dose, kidney irritation was noted in fourteen to twenty-eight days. Hemopoiesis and liver function were not disturbed. Hearing loss occurred in 2 cases.

The type of disease in most patients studied did not lend itself to fair clinical evaluation of efficacy. Roentgenographic improvement occurred in 4 cases. In 10 who received the drug for more than six weeks, symptoms were favorably altered in 4.

Fifty per cent of the tuberculous infections became kanamycin resistant within three months.

The place of kanamycin in the therapy of tuberculosis cannot be evaluated from these observations, but it is good enough to warrant further and extensive trials.

Corticosteroids in the Treatment of Tuberculosis

HASKELL J. WEINSTEIN and JOHN J. KOLER, Seattle, Wash.

One hundred patients examined consecutively with active pulmonary tuberculosis were randomly assigned to a strict double-blind controlled program of treatment with the usual antituberculous drugs plus prednisolone (Sterane®) tablets or an appropriate placebo. The patients were screened for the usually accepted contraindications to steroid therapy. The steroids were administered for sixty-eight days to a total of 780 mg. per patient. All of the patients were treated concomitantly with isoniazid and aminosalicylic acid or one of its derivatives. Multiple parameters were studied, including the usual roentgenographic and laboratory procedures as well as the clinical variables. No significant detrimental effects attributable to the steroids were noted in this study.

Highly significant acceleration of roentgenographic clearing was observed in the treated group, particularly in those with far advanced disease. Reversal of infectiousness occurred in a significantly higher percentage of the treated patients. In 16 of 51 treated patients only the admission sputum studies were positive for *M. tuberculosis*, whereas only 6 of 49 control patients showed reversal of infectiousness during the same period.

Twenty-four of 32 patients with cavitary disease in the treated group achieved cavity closure, 16 of these on the medical regime and 8 others with surgical intervention. Seven others achieved a consistent "open-negative" status and were discharged. Only one patient is still hospitalized with a cavity recognizable roentgenographically. Among the control

TABLE 1

NUMBERS OF COLONIES OF TUBERCLE BACILLI RECOVERED FROM GUINEA PIGS INFECTED WITH TUBERCLE BACILLI AND THEN TREATED BY DAILY ADMINISTRATION OF KANAMYCIN OR STREPTOMYCIN FOR SEVEN WEEKS

Treated with	Guinea Pigs	Lung	Organ	
			Liver	Spleen
Untreated control animals	1	3,300	7,600	3,300
	2	4,000	2,400	2,900
	3	3,300	8,000	2,000
	4	11,000	1,700	9,000
	6	5,000	2,300	2,000
	7	4,200	3,600	3,000
	8	31,000	5,000	1,500
	9	3,100	1,300	4,000
	10	116,000	11,300	36,000
Kanamycin, 10 mg. daily	21	0	0	21
	22	0	0	7,000
	23	0	0	3
	24	0	2	2,400
	25	0	10	26
	26	5	0	0
	27	0	140	10
	28	1	230	70
	29	32	250	190
	30	3	5	20
Streptomycin, 10 mg. daily	46	19	0	5
	47	7	0	570
	48	6	31	17
	49	2	7	1
	50	83	230	2
	51	6	0	12
	52	0	0	0
	53	0	0	13
	54	0	0	8
	55	0	0	0

Note: Numbers in the table indicate viable bacterial units recovered from 10 mg. of the tissue.

Drug administration was started two weeks after infection. Infection was made by intravenous route.

Guinea pig *No. 5* died shortly after the start of experiment because of accident.

group, 27 patients had cavitary disease. Cavity closure has been achieved in 15 of these, in 8 medically and 7 surgically. At the present time 9 of the control patients are still hospitalized with open cavitary disease while only 3 have achieved a consistent "open-negative" status permitting discharge. These results are highly significant statistically.

The findings in this study suggest that there may be significant benefit to be derived from combined steroid-antituberculous drug therapy in pulmonary tuberculosis, particularly for far advanced cavitary disease.

TABLE 2

NUMBERS OF COLONIES OF TUBERCLE BACILLI RECOVERED FROM GUINEA PIGS INFECTED WITH VARIOUS DRUG-RESISTANT TUBERCLE BACILLI AND TREATED BY DAILY ADMINISTRATION OF KANAMYCIN FOR 31 DAYS

Infected with	Treated with	Guinea Pigs	Organ		
			Lung	Liver	Spleen
Streptomycin-resistant tubercle bacilli	Streptomycin, 10 mg. daily	1	99	68	5,000
		2	8	208	4,500
		3	42	168	12,000
		4	8	11	3,300
		5	180	171	5,600
		6	112	23	4,500
		7	Contaminated	104	7,000
	Kanamycin, 10 mg. daily	15	0	4	990
		16	0	1	70
		17	0	2	33
		18	0	2	78
		19	2	3	360
		20	27	10	235
		21	21	4	540
Isoniazid-resistant tubercle bacilli	Isoniazid, 10 mg. daily	36	0	0	44
		37	0	0	75
		38	0	0	11
		39	0	0	3
		40	0	0	29
		41	0	0	72
		42	0	0	2
	Kanamycin, 10 mg. daily	43	0	0	0
		44	0	0	0
		45	0	0	1
		46	0	0	0
		47	0	0	0
		48	0	0	0
		49	0	0	0
PAS-resistant tubercle bacilli	PAS, 200 mg. daily	71	190	110	400
		72	20	70	1,300
		73	1	10	1,100
		74	30	30	2,100
		75	1	20	2,200
		76	20	70	2,000
		77	90	130	4,800
	Kanamycin, 10 mg. daily	78	0	0	8
		79	0	0	8
		80	0	0	3
		81	0	0	44
		82	0	1	62
		83	0	0	61
		84	1	0	43

Note: Numbers in the table indicate viable bacterial units recovered from 10 mg. of the tissue.

Drug administration was started one day after infection.

TABLE 3

VIABLE BACTERIAL UNIT COUNTS OF THE LUNG, LIVER, AND SPLEEN OF TUBERCULOUS GUINEA PIGS UNDER DIFFERENT THERAPEUTIC CONDITIONS

Treatment	Guinea Pig Number	Organ		
		Lung	Liver	Spleen
Pretreatment	1	65	73	960
	2	117	60	3,300
	3	15	160	2,900
	4	200	350	6,200
	5	7	310	730
Untreated	6*	1,800	8,500	11,000
	7*	98,000	19,000	72,000
	8*	7,200	4,000	9,300
	9	11,000	4,000	5,000
	10	13,000	12,000	5,200
Kanamycin, 20 mg. daily	11	0	0	0
	12	0	0	0
	13*	0	0	0
	14	0	0	0
	15	0	0	216
Streptomycin, 20 mg. daily	16	0	0	0
	17	0	0	4
	18	0	0	Contamination
	19	0	0	10
	20	5	4	124
Isoniazid, 20 mg. daily	21	0	0	0
	22	0	0	0
	23	0	0	0
	24	0	0	Contamination
	25	0	0	0

* Animals died before autopsy, each 121, 123, 93, and 145 days after infection. Counts in the table indicate viable units in 10 mg. of the tissue.

Treatment was started 6 weeks after infection and continued for 15 weeks.

Autopsy was made ten days after the termination of treatment.

The Effect of Increasing Oral Isoniazid Dosage on Biologically Active and Total Isoniazid Concentrations in the Blood

ROBERT L. TAYLOR, O. L. WEISER, RICHARD S. FRASER, JAMES A. WEIR, and RICHARD R. TAYLOR, Denver, Colo.

The metabolism of isoniazid to one or more biologically inactive derivatives has been demonstrated by several workers. Significant variation from person to person has also been established, making an intelligent prediction of free isoniazid serum concentrations impossible and each case an individual therapeutic problem. For these reasons the bioassay technique has been employed at this hospital for the past four years to adjust the dosage of isoniazid necessary to maintain what is thought to be an adequate concentration of biologically active drug until the sixth hour.

TABLE 4

INHIBITORY ACTION OF STREPTOMYCIN AND ISONIAZID ON THE MULTIPLICATION OF KANAMYCIN-RESISTANT TUBERCLE BACILLI IN GUINEA PIGS

(Viable Bacterial Unit Counts)

Treated with	Guinea Pig Number	Organ		
		Lung	Liver	Spleen
Kanamycin, 10 mg. daily	46	230	200	700
	47	520	230	1,200
	48	580	800	4,300
	49	19	75	300
	50	43	170	1,500
Streptomycin, 10 mg. daily	38	0	1	96
	39	0	1	400
	40	0	0	132
	41	0	0	600
Isoniazid, 10 mg. daily	42	0	2	2
	43	0	0	0
	44	0	0	0
	45	0	0	3

Note: Number indicates viable units in 10 mg. of the tissue.
Drug administration was started three days after infection and continued for 28 days.
Autopsy was made seven days after the termination of drug administration.

An experiment was designed to determine the effect of increasing dosages of isoniazid on total and free serum isoniazid. Nineteen patients with active tuberculosis were primed for two days on 4 mg. per kg. per day (one-third three times daily) of oral isoniazid and were bled two hours after the morning dose, which corresponds to the peak serum concentration, and the sixth hour which shows the effect of metabolism and excretion. Free isoniazid serum concentrations were determined biologically and total isoniazid, biologically active and inactive, serum concentrations were determined chemically.

The above procedure was repeated, using dosages of 8 mg. per kg. per day (one-third three times daily), 16 mg. per kg. per day (one-third three times daily), and 24 mg. per kg. per day (one-third three times daily). The chemically determined total isoniazid concentrations, at two and six hours, were found to be at least double with each twofold increase in drug. The biologically determined concentrations of active drug at the second hour near peak level were found to double with each twofold increase in drug as were the six-hour concentrations after a detectable concentration had been obtained.

These results indicate that an intelligent prediction of free serum isoniazid can be made after an initial bioassay determination and may prove valuable to clinicians whose access to the bioassay procedure is limited.

Cycloserine and Viomycin in the Treatment of Pulmonary Tuberculosis

WILLIAM S. SCHWARTZ and RALPH E. MOYER, Oteen, N. C.

Cycloserine, 0.25 gm. twice daily by mouth (0.5 gm. daily) and viomycin, 1 gm. twice daily intramuscularly, twice weekly (2 gm. twice weekly) have been given to 57 patients with infectious sputum who were suffering from moderately or far advanced pulmonary tuberculosis. All but one had demonstrable pulmonary cavities. All were treatment failures

following the use of other drug regimens, surgical procedures when applicable, and bed rest. None had received cycloserine or viomycin previously.

Treatment of the first patient was begun on June 2, 1957. Twelve patients have been lost to the study: 4 because of toxicity; one, refusal of treatment; 5, unsanctioned discharge; 2 died. Toxicity consisted of gastrointestinal and hypersensitivity phenomena. One death was due to cor pulmonale without progression of pulmonary disease. The other occurred during a surgical procedure.

Fever disappeared in 6 patients who had been febrile before treatment. Twenty patients gained weight, 2 lost weight, and the rest remained the same.

A review of serial roentgenograms prior to treatment of those who received the regimen for at least one month revealed the pulmonary disease to have been progressive in 8 and stationary in 43. The results following treatment were about what might have been expected from any drug regimen in such an unfavorable group of cases. Those patients who had shown unfavorable progression previously showed clearing of the recent disease, while those who had had no change for quite some period of time before treatment showed little change during treatment. Only one patient showed evidence of unfavorable roentgenographic progression during treatment.

The sputum of a high proportion of the patients became negative for acid-fast bacilli on microscopy rather promptly, but the sputum of most of the patients has remained positive for *M. tuberculosis* on culture up to the present time.

Very preliminary data regarding bacterial resistance are encouraging in that no definite evidence of increased bacterial resistance to either drug has appeared.

It is believed that at present it may be concluded, tentatively, that the regimen of cycloserine and viomycin shows promise of being more useful as a secondary regimen not involving major drugs than any of this type we have tried previously.

Pregnancy Following Major Thoracic Surgery For Tuberculosis

GEORGE SCHAEFER, STANLEY J. BIRNBAUM, and R. GORDON DOUGLAS, New York, N. Y.

Eleven per cent of 257 tuberculous patients delivered at The New York Lying-In Hospital between 1952 and 1956 had major thoracic operations for pulmonary tuberculosis performed before or during pregnancy. The majority of the operative procedures were resections performed before conception. Three operations were done during pregnancy and one shortly after delivery.

Labor was managed with small repeated doses of Demerol®. Narcotic doses of drugs which depress the cough reflex and voluntary expectoration were avoided. Local or pudendal block was the anesthesia of choice for delivery, which was made as simple as possible for the patient. This entailed careful evaluation of each patient and the use of low forceps delivery to shorten the second stage of labor if this was prolonged or tiring. A longer period of postpartum rest was advised and breast feeding was not permitted.

The results following the 32 deliveries in 27 patients showed no change in 25 instances, improvement in 5 patients in whom the disease was active during pregnancy, and progression in 2 instances. Follow-up was from five to seventy-three months after delivery.

Conclusions

1. Pregnancy may be safely undertaken following major thoracic surgery for tuberculosis if the patient receives adequate medical and obstetrical care.

2. While major thoracic surgery may be safely performed during the first and second trimesters of pregnancy, we believe it is best undertaken in the nonpregnant state except in unusual circumstances.

3. Antimicrobial therapy should be given to the pregnant patient before, during, or after gestation in the same manner as it is to the nonpregnant patient.

4. Respiratory embarrassment has not been a frequent occurrence during pregnancy and labor in the patient who has undergone major thoracic surgery.

Tuberculous Polyserositis

CHARLES G. SPIVEY and JAMES A. WEIR, Denver, Colo.

Data are presented on review of 25 patients with proved or probable tuberculous polyserositis discharged from the Tuberculosis Service, Fitzsimons Army Hospital, between 1953 and 1956. These patients all had involvement of multiple serous cavities, to include bilateral pleurisy with effusion. Few of them had definite pulmonary tuberculosis. Proved miliary tuberculosis was present in several.

A majority of these cases occurred in non-white persons such as Negro, Japanese Americans, and so forth. Most occurred in young adults (no pediatric cases are included).

Comment is made concerning difficulty of diagnosis in this syndrome, particularly in differentiation from cases of possible collagen or collagen-like diseases such as rheumatoid arthritis. Mention is made of 5 cases of probable nontuberculous polyserositis to focus attention on problems of differential diagnosis.

A brief review of the literature and of current thinking on etiology and pathogenesis of this syndrome is presented. The treatment of the 25 cases of tuberculous polyserositis is discussed.

Pelvic Tuberculosis in the Female

ROBERT R. HENDERSON, JULIA M. JONES, and GORDON W. DOUGLAS, New York, N. Y.

Tuberculosis of the female reproductive system has received relatively little attention because diagnostic methods have been unreliable. During the period from 1954 to 1957, 25 cases of this type have been admitted to the chest or gynecological services of Bellevue Hospital. As a part of a combined gynecological-medical study, these patients have been treated by protocol, with careful pathologic and bacteriologic study of biopsied and resected specimens.

An attempt has been made to evaluate the efficacy of diagnostic methods, characteristics of the clinical course, and effect of combined medical-surgical management. After pathologic or bacteriologic diagnosis, all patients were started on a similar course of antituberculous chemotherapy for a period of at least one year. Some patients, with more complicated or acute disease, were transferred to Ray Brook Hospital for prolonged hospital care; but the majority were followed by us in the clinic. After various intervals of chemotherapy, extirpation of pelvic organs was performed in a majority of the patients. In the others, without palpable pelvic residua, serial endometrial biopsies were obtained as an integral part of the follow-up study.

Of the cases reported, all but 4 have been followed for intervals of 6 to 48 months after the onset of therapy. The average follow-up observation was 28.5 months. The other 4 cases were lost to the study at the end of an average of 8 months due to social or environmental factors. All 25 patients were doing well at the time of the last examination, including those lost to the study. Only 5 cases offered evidence of active tuberculosis in other areas in spite of complete evaluation during admission. Six patients gave a history of old tuberculous infection and, of these, 3 had had pleural effusion.

Kodachrome slides representative of the gross and microscopic changes in resected specimens, after varying lengths of chemotherapy, are presented.

1B Physiology

Respiratory Function in Pulmonary Tuberculosis

DAVID G. SIMPSON, JOHN H. MCCLEMENT, and ANDRE COURNAND, New York, N. Y.

Patients with active pulmonary tuberculosis have been classified according to the extent, character, and duration of the disease; and to the effect of chemotherapy. Efforts have

been made to describe the extent and nature of the disease in the most precise terms in order to facilitate accurate comparison. When resection of residual disease was carried out, the result of pathologic study of the specimens was used to enhance this correlation. Physiologic studies include lung volumes and ventilatory capacity, ventilation, blood gases using oxygen saturation at rest and on exercise, and oxygen tension studies with calculation of the dead space, venous admixture, and diffusing capacity. The findings in the different groups of patients are compared, and the changes in patients studied on two or more occasions during treatment are presented.

The Prevalence of Diffuse Airway Obstruction in Men Between 40 and 65 Years of Age

GEOFFREY L. BRINKMAN and E. OSBORNE COATES, JR., Detroit, Mich.

This is a preliminary report on a study to assess the prevalence of diffuse airway obstruction in an industrial population. So far, 302 men, 40 to 65 years old, have been studied by questionnaire, spirogram (including vital capacity, 1-, 2-, and 3-second vital capacity, and maximal mid-expiratory flow), and inspiratory and expiratory chest roentgenograms.

The one-second vital capacity has proved of no diagnostic use due to its wide scatter. The 3-second vital capacity showed less scatter and, if 90 per cent is accepted as the lower limit of normal for this age group, then there is an increasing incidence of diffuse airway obstruction manifest. In the age group 40 to 44, 11 per cent of the subjects had a 3-second vital capacity below 90 per cent. The incidence of diffuse airway obstruction steadily increased so that in the age group 60 to 65, 41 per cent had a 3-second vital capacity below 90 per cent.

The maximal mid-expiratory flow showed a similar trend. If 2.25 liters per second is accepted as the lower limit of normal, then 7 per cent of the subjects 40 to 44 years old, increasing to 50 per cent in the group aged 60 to 65, have a maximal mid-expiratory flow below 2.25 liters per minute. The correlation between the 3-second vital capacity and the maximal mid-expiratory flow is very good for the low values, but there is some discrepancy in the borderline area between normal and abnormal values.

Diaphragmatic movement was correlated with the 3-second vital capacity, and it was found that there was no relationship between the range of movement of the diaphragm and the 3-second vital capacity.

There was a high incidence of bronchitis in this group, and there was a definite relationship between the number of cigarettes smoked and the incidence of bronchitis. Those men who had bronchitis had a considerably increased incidence of diffuse airway obstruction.

Observations on the Circulatory Dynamics in Patients with Chronic Lung Disease

KAYE H. KILBURN and H. O. SIEKER, Durham, N. C.

Although patients with chronic obstructive pulmonary emphysema are usually easily distinguished from those with congestive heart failure, subjects in whom these two conditions are combined present diagnostic and therapeutic problems. To investigate the relationships between the pulmonary and cardiac insufficiency observed frequently in these patients, the effects of certain mechanical and pharmacologic stimuli on the heart and lungs were studied. Pulmonary arterial pressure, "wedge" pressure, cardiac output, and blood gases were determined at rest, during exercise, during 100 per cent oxygen breathing, and during the infusion of isoproterenol and acetylcholine. Results in 10 patients with chronic lung disease and 8 patients with heart disease primarily are compared with a normal control group.

In contrast to normal subjects, exercise raises pulmonary arterial pressure (mean difference, 11 mm. of mercury), increases cardiac output slightly (mean difference, 0.27 L/min/sq. m.) and decreases blood oxygen saturation in patients with chronic lung disease. Breathing 100 per cent oxygen lowers pulmonary arterial pressure (mean difference, 3.5 mm. of mercury) without affecting cardiac output. Isoproterenol raises pulmonary arterial pressure (mean difference, 3.2 mm. of mercury) and increases cardiac output (mean difference, 0.77 L/min/sq.m.). In patients with heart failure, isoproterenol lowers pulmonary arterial and

"wedge" pressure while increasing cardiac output. Acetylcholine infusion, which decreases pulmonary arterial pressure without changing pulmonary "wedge" pressure in normal subjects and subjects with congestive heart failure, likewise produced an early drop in patients with chronic lung disease; but prolonged infusion raised pulmonary arterial pressure without altering cardiac output.

Patients with chronic lung disease cannot decrease the pulmonary vascular resistance in response to mechanically or pharmacologically induced increases in cardiac output. They can decrease pulmonary vascular resistance in response to an increased oxygen tension of the blood. These findings suggest that the pulmonary vessels serve as relatively nondeformable transfer tubes during left ventricular failure and complement the clinical observation that symptoms of right-sided cardiac failure predominate in patients with chronic lung disease and heart failure.

Body Potassium and Sodium and Extracellular Fluid in Chronic Pulmonary Insufficiency

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Published data obtained in the Pulmonary Function Unit of the Veterans Administration Hospital, Coral Gables, Florida, indicate unusual sensitivity to digitalis in chronic cor pulmonale. Cellular potassium and hypoxia have been incriminated in digitalis intoxication. Total body electrolyte composition and extracellular fluid volume were carried out by radioisotope-dilution techniques, as modified by one of us. Coincident with the final blood collection for the above, arterial blood was collected and measured by standard techniques. Ventilatory function and lung volumes were performed by methods described elsewhere.

The mean total body exchangeable potassium (TBK) in 42 subjects was 32.5 mEq per kg. (S.D. = 7.18) as contrasted with a normal of 46.3 mEq per kg. (S.D. = 4.31) ($P < 0.01$). Mean total body exchangeable sodium (TBNa) in 37 patients was 50.3 mEq per kg. (S.D. = 9.5) contrasted with a normal of 41.9 mEq per kg. (S.D. = 4.7) ($P < 0.001$). Mean extracellular fluid volume (ECF), using radiosodium, measured simultaneously with TBK and TBNa in 22 subjects was 232.7 cc. per kg. (S.D. = 67.4) contrasted with a normal of 162.6 (S.D. = 17.3) ($P < 0.001$). Values obtained by the modification by one of the authors agreed very closely with those obtained using the methods described elsewhere.

Correlative measurements in 34 subjects indicate poor correlation between TBK and TBNa and the differences between observed body weights and estimated average weights (Metropolitan Life Insurance Tables, 1943).

There was a significant linear correlation between calculated arterial carbon dioxide tension and TBNa ($r = 0.4147$) ($0.02 > P > 0.01$). TBNa correlated poorly with calculated arterial oxygen tension and pH; as did TBK and ECF with the arterial blood determinations. Apparent total body intracellular sodium was calculated in 19 subjects and likewise bore no relationship to any of the arterial blood measurements.

Only four of these 34 subjects were in right-sided failure at the time of the study.

In 29 subjects, 1.2 mg. of acetyl strophanthidin were injected intravenously immediately after the arterial blood collection. Eight subjects exhibited electrocardiographic evidences of sensitivity. The mean arterial oxygen tension for the sensitive patients was significantly lower than that for the nonsensitive subjects ($P = 0.01$). The mean TBK and pH were lower for the sensitive group than for the nonsensitive group, whereas the mean TBNa and arterial carbon dioxide tensions were higher for the sensitive group. However, none of these latter differences between the sensitive and nonsensitive groups were statistically significant at the 10 per cent level ($P > 0.1$).

Conclusions

Significant ($P < 0.01$) depletion of body potassium, sodium retention, and expansion of extracellular fluid volume were noted in this group of patients with chronic pulmonary

insufficiency. There was a significant correlation between increase in exchangeable body sodium and the arterial carbon dioxide tension. Sensitivity to digitalis is most apt to occur with the more severe grades of hypoxia, and may also be associated with potassium depletion, carbon dioxide retention, and lowering of arterial pH.

Multiple Pulmonary Air Cysts

GUSTAVE A. LAURENZI, GERARD M. TURINO, and ALFRED P. FISHMAN, New York, N. Y.

During the course of study of patients with multiple pulmonary air cysts, it became evident that many failed to show the clinical and physiologic characteristics generally identified with chronic obstructive pulmonary emphysema. A separate study was undertaken to delineate the characteristics of this group. For this presentation, the term "air cyst" will be used to indicate any air-containing lesion of the lung disclosed by roentgenographic examination; by this use, it is intended to avoid any implication with respect to anatomic structure, specific location, or pathogenesis.

Fifteen patients with multiple large air cysts of both lungs were studied by: (*1*) measurement of lung volumes; (*2*) assessment of gas exchange, including estimation of the diffusing capacities of the lung for oxygen and carbon monoxide; (*3*) determination of pulmonary arterial blood pressures and flows by the technique of right heart catheterization; (*4*) bronchospirometry; and (*5*) application of specialized roentgenographic techniques, including tomography, bronchography, and angiocardiography.

The results indicate that two distinct groups of patients with large, bilateral cysts may be identified on clinical and physiological grounds: those *without* diffuse bronchial obstruction, and those *with* diffuse bronchial obstruction who show all the characteristics of chronic obstructive emphysema.

The group with unobstructed multiple air cysts gave no history of cough or bronchitis, and measurements of the vital capacity, maximal breathing capacity, index of intrapulmonary mixing, and the ratio of residual volume to total lung capacity were all normal. Single-breath and continuous nitrogen washout curves, however, indicated a definite abnormality in the distribution of inspired gas throughout the lung. In the group with emphysema, cough and bronchitis were prominent and were associated with physiologic disturbances characteristic of diffuse bronchial obstruction.

Both groups showed abnormalities in ventilation-perfusion relationships as well as the occurrence of pulmonary arterial hypertension during exercise. In general, the derangements were more prominent in those with obstructive emphysema.

The physiologic characteristics of the group without generalized bronchial obstruction may be obscured during an acute respiratory infection or following repeated attacks of bronchitis and pneumonia. Even at this stage, differentiation may be possible by careful analysis of the history and by repeated tests of pulmonary function.

Relationship Between Airway Resistance and the State of Inflation of the Lung in Normal Subjects and in Patients with Obstructive Disease

TSUNG O. CHENG, M. P. GODFREY, and RICHARD H. SHEPARD, Baltimore, Md.

The relationship between airway resistance and the state of inflation of the lung was estimated throughout the expired vital capacity using the multiple interrupter of Clements and Elam and a servo-spirometer. In normal subjects the airway resistance was lowest near full inflation and remained relatively constant until about 80 per cent of the vital capacity had been expired. It then rose abruptly and approached infinity at full expiration. These results are similar to those found by Briscoe and DuBois using another method. In contrast, patients with airway obstruction exhibited the abrupt rise in resistance much closer to the position of maximal inspiration, although the resistance values prior to this were not necessarily elevated.

In both normal subjects and in patients with obstructive disease, the values for airway resistance approached infinity at full expiration; and it would therefore appear that in both groups the total volume of air which can be expired is limited by closure of the airways. In assessing the significance of airway resistance during expiration, it is important to determine the relationship between that resistance and the state of inflation of the lung.

The Work of Breathing with Increased Airway Resistance and Intermittent Positive Pressure Assistance

ROBERT H. JONES, JAMES MACNAMARA, MARJORIE F. ELLICOTT, and EDWARD A. GAENSLER, Boston, Mass.

Increasing recognition that under-ventilation must be treated by artificial respiration has led to extensive use of intermittent positive pressure breathing (IPPB) devices. Evaluation of its effectiveness in patients with severe acute respiratory acidosis and coma is difficult because experimentation in very ill patients is intolerable and results often unreliable.

Consequently, we have made a device for external obstruction to air flow in which the resistance increases in proportion to the intrapleural pressure. This valve can be adjusted so that the pneumotachogram of normal subjects mimics that of patients with severe chronic obstructive emphysema. A number of measurements have been made both at rest and during IPPB therapy. Changes in pneumotachogram, respiratory rate, alveolar ventilation, work of breathing, airway pressure, and pCO_2 were made.

Preliminary results suggest that under certain circumstances IPPB may actually increase the work of breathing and hypercapnia and diminish alveolar ventilation. Clinical experiences are related which parallel these experimental observations.

The Pulmonary Diffusion Capacity for Carbon Monoxide

M. MOSTAFA ABDEL-FATTAH, MARGARET E. GANTT, and DAVID W. CUGELL, Chicago, Ill.

A variety of methods for determining the pulmonary diffusing capacity for carbon monoxide (D_LCO) has been devised. The continuous breathing methods are most suitable for studies during exercise. We have adopted the method of Bates which obviates the need for an arterial puncture. The alveolar carbon monoxide tension is calculated from an assumed value for the expiratory dead space or by automatic end-tidal sample collections.

The following information has been obtained:

1. Erroneous estimations of the dead space have little influence upon the calculated D_LCO if the tidal volume dead-space ratio is relatively large. A family of curves has been constructed to define the limits of this ratio for a reliable result over the usual range of inspired/expired carbon monoxide concentrations.
2. During moderate exercise, end-tidal samples are probably representative of the mean alveolar carbon monoxide concentration, but during strenuous exercise they are not and yield falsely high diffusion capacity values.
3. In normal subjects, three to four determinations at thirty-minute intervals are possible before a significant decrease in D_LCO occurs due to carbon monoxide accumulation.
4. Hyperventilation, increasing the tidal volume with normal minute ventilation, and carbon dioxide inhalation do not alter the D_LCO significantly.

Relationship Between Pulmonary Capillary Blood Flow, Pulmonary Diffusing Capacity, and Pulmonary Capillary Blood Volume at Rest and Exercise

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Previous studies on the pulmonary diffusing capacity (D_L) in man (Ogilvie *et al*, *J. Clin. Investigation*, 36: 1, 1957), by the CO breath-holding technique, disclosed that not

only did it increase during exercise presumably secondary to changes in pulmonary capillary blood flow (Q_C), but that these changes could take place in a matter of seconds. This suggested that the proper interpretation of measurements of D_L requires simultaneous measurements of Q_C. Therefore we have developed a technique for measuring D_L and Q_C simultaneously. This consists of inspiring a gas mixture containing about 0.3 per cent CO, 0.4 per cent acetylene, 10 per cent helium, 20 per cent O_2, and the remainder N2, holding this breath for a measured time, and then delivering an expired alveolar gas sample.

D_L is calculated from the disappearance of CO relative to helium, and Q_C from the disappearance of acetylene relative to helium. If the alveolar O_2 concentrations are varied, pulmonary capillary blood volume (Vc) can be calculated from the different estimations of D_L. Q_C during Valsalva maneuver, at rest, and at the highest level of exercise in 4 normal subjects was 3,260, 5,960, and 14,000 ml. per min., respectively; corresponding values of D_L were 21, 26, and 44 ml. per (min. × mm. of mercury), respectively. D_L and Q_C were linearly related in an individual subject and showed no maximal plateau. Vc was measured in 2 of these subjects and was also linearly related to Q_C.

2A Surgery

Pleural Tuberculosis: A Clinicopathological Study

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New York, N. Y.

Utilizing the technique of pleural biopsy, pleural disease—tuberculous and nontuberculous—has been studied in more than 200 patients. Many of these have undergone more than one biopsy procedure, and several have been followed serially with regularly repeated biopsies during the entire known time span of their pleural disease. The pleural fluid in a large group of patients has been subjected to intensive study and, ultimately, a small group has had either open pleural biopsy or exploratory thoracotomy.

In this presentation, only the observations in tuberculous pleural disease will be considered. At least five patterns of disease have been identified histologically in tuberculous pleural tissue: acute, edematous, hypercellular pleura; granulomata, noncaseating in character; classical caseating tubercles; confluent caseation with associated fibrosis, hyalinization, and calcification; hyalinized and fibrous whorls of tissue.

Examples of these various patterns are demonstrated and clinical correlations presented.

Five or more serial biopsies have been performed at various intervals on each of several patients with tuberculosis who presented with pleural effusion. In the majority of these we were able at the outset to demonstrate caseating granulomata and, at the time of their final biopsy specimen, healing, as seen in beginning fibrosis, hyalinization, or scar formation. The time interval during which these changes occurred has been surprisingly short. All except the inital specimen were obtained during antituberculous chemotherapy. The uniformity of the histologic pattern at any time has been studied by comparing multiple specimens removed from multiple sites at the same time.

The material that has been collected in this study suggests that in pleural tuberculosis the pleura is diffusely involved by the characteristic pathologic spectrum of tuberculosis, and there is little to support the former concept that tuberculous pleural effusions are due to allergic phenomena.

With respect to those cases in which pleural biopsy failed to provide a definitive diagnosis, it is necessary to emphasize that pathology reports of nonspecific pleuritis do not exclude the diagnosis of tuberculosis. This series includes a small number of patients with the following pleural diagnoses: hyalinization and fibrosis, subacute pleuritis, dense pleural fibrosis, nonspecific pleuritis, calcific pleuritis, collagen, and noncaseating granulomata. In each of these the diagnosis of tuberculosis was eventually established either bacteriologically or at open thoracotomy. In an equal number whose specimens were characterized by nonspecificity, we have excluded the diagnosis of tuberculosis with fair assurance, because both the bacteriology and the tuberculin reactions were negative.

An Analysis of Preoperative Bronchial Biopsy in 100 Patients with Pulmonary Tuberculosis

NEIL C. ANDREWS, CLARENCE I. BRITT, and PHILIP C. PRATT, Columbus, Ohio

Many surgeons have depended on bronchoscopic examination as an indication of the presence or absence of tracheobronchial inflammation in patients with pulmonary tuberculosis. To study the reliability of gross bronchoscopic findings, 100 patients at the Ohio Tuberculosis Hospital have been bronchoscoped and the lobar bronchus draining the area of active disease biopsied. After this study, surgical resection was performed and the proximal portion of the resected bronchus was studied and compared with the bronchial biopsy. The gross bronchoscopic observation, the histologic studies of the biopsy, and the surgically resected bronchus have been correlated with the incidence of postoperative complications in these 100 patients.

As has been shown previously, there is a definite relationship between the presence of submucosal tubercles or lymphocytic infiltration in the bronchus and the incidence of complications of bronchial origin following resection. This study shows that the bronchoscopic appearance of the mucosa does not reflect the condition of the bronchus, and only by biopsy can the presence of submucosal inflammation be ascertained.

No complications have been associated with the use of bronchial biopsy in patients with pulmonary tuberculosis.

Pneumonectomy in the Treatment of Pulmonary Tuberculosis

T. W. SHIELDS, W. M. LEES, and R. T. FOX, Chicago, Ill.

The surgical resection of residual or persistent disease after adequate medical treatment has achieved a firm place in the management of the individual with pulmonary tuberculosis. Excellent early as well as late results have been obtained following subsegmental, segmental, and lobar resections. However, the morbidity and mortality of pneumonectomy has remained high. The long-term follow-up studies of patients surviving pneumonectomy have revealed various results.

An analysis of 101 patients who underwent pneumonectomy as a part of the treatment for pulmonary tuberculosis at the Chicago Municipal Tuberculosis Sanitarium during the years 1951 through 1955 is presented.

The indications for pneumonectomy were destroyed lung in 34, extensive disease throughout the lung in 32, thoracoplasty failure in 26, bronchiectasis in 2, cystic disease in one, massive pulmonary hemorrhage in one, and technical reasons in 5 patients. The sputum was positive for tubercle bacilli in 44.5 per cent, and chemotherapy had been given in interrupted courses in 40.5 per cent.

Eleven patients died within sixty days of the procedure, a postoperative mortality of 10.8 per cent. An additional 5 patients (4.9 per cent) died within one year as a result of either the complications or sequellae of the operation. Eighteen patients developed one or more major nonfatal complications; in only 2 patients were these nontuberculous complications. Fifty-seven patients had uneventful postoperative courses.

Cardiorespiratory failure was the most common nontuberculous complication. Bronchopleural fistula and spread to, or reactivation of, contralateral disease were the most serious fatal and nonfatal tuberculous complications. The former occurred in 8.9 per cent and the latter in 15.8 per cent of the patients. The presence or combination of an acute endobronchitis, sputum positive for tubercle bacilli, and interrupted chemotherapy was significant in these two groups.

Of the 85 patients surviving for more than one year, 37 were discharged with inactive disease; 18 with arrested disease; 11 with active improved disease with a good prognosis; 8 with active disease, maximum hospital benefit; 6 patients were discharged against medical advice; and 2 died after a prolonged hospital stay of more than 24 months postoperatively.

A two- to seven-year follow-up study of the patients surviving for more than one year

is in progress. To date it is 74 per cent complete. Of these 61 patients, 49 are classed as having inactive disease, 4 with active disease, and 8 have died.

The Collapse Therapy of Pulmonary Tuberculosis by Means of Extraperiosteal Plombage

THOMAS F. BOYD, EUGENE G. LAFORET, and JOHN W. STRIEDER, Boston, Mass.

Pulmonary resection is the commonest operation performed for pulmonary tuberculosis in our clinic at the Sanatorium Division of the Boston City Hospital. In this modern chemotherapy era, resection has become a safe operation, although it has an appreciable mortality and morbidity. However, in the same era, collapse surgery, which was believed to be safe prior to the advent of drugs, has become even safer and more effective.

A study has been made of all patients who had extraperiosteal plombage thoracoplasty performed at the Sanatorium Division of the Boston City Hospital from February 22, 1949 (the date of the first such operation) through December 31, 1956. This allows time for a follow-up study of fifteen months to nine years.

In general, this procedure was selected for the older patient, for the patient with a poor functional reserve, or for the patient with drug-resistant tubercle bacilli in the sputum. Frequently, it was used for the patient with all three of these poor prognostic factors present. In this latter group of patients, that is, the older patient with poor pulmonary function, a drug-resistant infection, and possibly bilateral disease, this is in our judgment the only possible surgical procedure which may be considered.

One hundred and forty-nine operations have been performed: 129 patients have had unilateral collapse procedures; and 10 patients have had bilateral plombage procedures. Of those with unilateral plombage, 67.5 per cent had sputum "conversion" and were discharged from the sanatorium as arrested cases. Of those with bilateral plombage, 50 per cent had sputum "conversion" and were discharged as arrested cases.

There were only two early postoperative deaths. These, as well as the late deaths and other complications, such as subscapular space infection, are discussed in detail.

Follow-up studies are also reported.

Resectional Therapy for "Open-Negative" Tuberculous Lesions

WATTS R. WEBB and JESSE L. WOFFORD, Jackson, Miss.

The classification of residual tuberculous lesions according to their bacillary and cavitary status can lead to a more objective approach in deciding which need resection. The presence or absence of a residual cavity seems to be the most important factor in the final result of antituberculous therapy.

To compare the results of resection of original treatment "open-negative" cases with re-treatment cases, 107 consecutive resections in 102 patients performed in the three years since January 1, 1955, have been analyzed. Only those patients are included who had pathologic demonstration of cavitation and sputum negative for tubercle bacilli during the two months immediately before operation. Bronchiectatic lesions are not included. Sixty-nine are "original therapy" and 38 are "re-treatment" cases. The re-treatment group had a higher proportion of Negroes and required more extensive resections with only one segmental and one wedge resection, but 16 pneumonectomies and 20 lobectomies. The original therapy group included 7 pneumonectomies, 42 lobectomies, 19 segmentals, and one wedge resection.

One early and one late death occurred in each group, only the late re-treatment death being due to tuberculosis. There were no early spreads and only one bronchopleural fistula. The incidence of other complications in the two groups was low and almost identical. Two patients from each group have since shown bacteriologic, roentgenographic, or combined activity, and the disease in 2 of these is now inactive.

Thus, of 102 patients, 98 are living, 2 with disease and 96 free of all active disease. These

observations demonstrate the resections can be performed for "open-negative" tuberculous lesions with satisfactory results in either the original or re-treatment group. In both groups the results are far superior to the published results of long-term medical therapy, and suggest that residual cavities, regardless of noninfectious sputum, should be resected.

Tracheal Fenestration as a New Method of Treatment for Advanced Emphysema

EDWARD ERNEST ROCKEY, SAMUEL ALCOTT THOMPSON, CHARLES FRANCIS BLZSIK, EDGAR MAYER, and ISRAEL RAPPAPORT, New York, N. Y.

Tracheal fenestration is a new conception in the treatment of emphysema. The operation creates a skin-lined tracheocutaneous opening guarded with airtight external skin valves. It provides a means of ready and repeated access to the tracheobronchial tree. The outer end of the tube has two skin valves which are in close apposition and prevent leakage of air or liquid. These valves may be manually opened for aspiration and medication. When not in use, the valves are in contact and the normal tracheal air tract is re-established. It does not affect either phonation or the cough mechanism. The skin tube is formed from two opposing skin flaps over the mid-part of the anterior surface of the neck. The inner end of the tube is attached to the cervical trachea through a window which is produced by partial excision of two neighboring tracheal cartilages and the intercartilagenous tissue.

Tracheal fenestration has been performed to date on 6 patients. Four of them had advanced pulmonary emphysema. Two of the 4 patients had suppurative emphysema with considerable cough and expectoration; 2 of them had dry emphysema without cough or expectoration; all of them had completely disabled dyspnea even at rest. Catheter suctionings led to effective elimination of the occluding, thick, tenacious bronchial secretion from all of these patients. Two of the 4 patients improved sufficiently to leave the hospital for the first time in years.

The fact that even from the patients with dry emphysema a considerable amount of thick, doughy, tenacious bronchial secretion was removed by aspiration indicates that the main source of the respiratory disability in both wet and dry emphysema is secretional occlusion rather than bronchial spasm.

Reconstructive Surgery of the Trachea and Bronchi

OSLER A. ABBOTT and EVERTON SANTOS, Emory University, Ga.

An analysis is made of lesions requiring consideration for tracheal or bronchial reconstructive surgery. The methods available include: (*a*) plastic suture repair, (*b*) dermal grafts, (*c*) excision of tracheal lining and grafting (*d*) anastomotic procedures, and (*e*) newer tracheal reconstructive procedures.

Analysis of our experiences reveals that:

1. Plastic suture repair has limited but definite usefulness.
2. Dermal grafts—
   a. can be used successfully in children.
   b. allow excisional surgery in lung carcinoma patients with restricted pulmonary reserve.
   c. permit radical right pneumonectomy, carinal excision, partial excision of trachea and left bronchus in selected cases.
   d. function poorly when used to replace cartilage ring containing areas of the cervical trachea.
3. Excision of tracheal lining and grafting can be used for uncontrollable papillomatosis of the trachea.
4. Anastomotic procedures have a wide usefulness within the thorax but are only useful in the cervical trachea wherein no major loss of tracheal substance has occurred.
5. A new method of a skin graft incorporating plastic tracheal rings is now available for reconstruction of massive cervical tracheal defects.

A short motion picture demonstrates the new two-stage procedure which we have devised for replacement of major areas of the cervical trachea.

The Value and Risks of Modified Needle Biopsy for Undiagnosed Lung Lesions

VINCENT W. LAUBY, W. EMORY BURNETT, GEORGE P. ROSEMOND, H. TAYLOR CASWELL, and R. ROBERT TYSON, Philadelphia, Pa.

Unfortunately, a significant percentage of pulmonary lesions remain undiagnosed even after thorough and complete study. In spite of careful clinical and laboratory studies—including roentgenograms of the chest, planigrams, bronchoscopy, bacteriologic and cytologic studies of the sputum, and scalene lymph node biopsy—the exact nature of the patient's pulmonary lesion remains undiagnosed. Exploratory thoracotomy becomes necessary.

The authors believe that there is a distinct advantage in having a definite diagnosis prior to thoracotomy and that there are definite disadvantages to incisional biopsy for diagnosis at the time of definitive surgery. In order to establish a definite diagnosis prior to thoracotomy, the technique of needle aspiration biopsy has been developed. The authors' experience began in 1936. Since that time, more than 600 needle aspiration biopsies have been performed on more than 500 patients.

In the paper, the rationale, the indications and contraindications, the technique, the results, the limitations, and the complications of the procedure are presented.

The paper, in its conclusions, shows that modified needle aspiration biopsy of pulmonary lesions remaining undiagnosed after conventional studies has a definite place in the clinician's diagnostic armamentarium. In properly selected cases, a definite diagnosis can be established prior to thoracotomy in a significant percentage without undue risk.

2B Immunity in Tuberculosis

Cellular Immunity in Tuberculosis: A Critical Evaluation of Some Observations

EMANUEL SUTER, Gainesville, Fla.

On the basis of available experimental results, it appears impossible to decide on any single mechanism to be responsible for infection immunity in tuberculosis. Many observations suggest that the mononuclear phagocyte of the immunized host is an important factor in this response. To test this further, attempts were made to transfer infection immunity from vaccinated donors to normal recipients of the same species by means of lymph node and peritoneal exudate cells. Collected cells were injected repeatedly into the peritoneal cavity or the veins. Control animals received cells from normal donors. Different criteria for successful passive immunization were used, and the results obtained are as follows:

1. Monocytes collected from recipients and infected with tubercle bacilli and cultured *in vitro* restricted intracellular multiplication of tubercle bacilli.
2. Recipient mice were injected with an attenuated strain R1Rv. The extent of the disease several weeks later was much smaller than in controls.
3. The course of a virulent infection with the Vallée strain was not altered in recipients of cells from vaccinated animals.
4. Intradermal lesions in the guinea pig which developed after injection of varying dilutions of R1Rv were smaller and healed more quickly in recipients of cells from vaccinated donors than in controls.

These results show that washed cells from vaccinated animals can induce a limited degree of infection immunity. The limited protection suggests that a complex mechanism is responsible for acquired resistance in tuberculosis. Experiments by others using serum from vaccinated animals point in the same direction.

The Interplay of an Immune Substance (Possibly Lysozyme) with Tuberculopolysaccharide and Its Antibody in Tuberculosis

FLORENCE B. SEIBERT, Philadelphia, Pa.

Antibodies were found not to have a direct bacteriostatic or bactericidal effect on finely dispersed BCG organisms *in vitro*. Instead of this, the antipolysaccharide antibody was

shown to play a possibly important role in immunity by combining with free circulating tuberculopolysaccharide, and so diverting it from occupying the effective groups of a naturally occurring bacteriostatic agent, thus leaving them free to act on the tubercle bacillus. Such a bacteriostatic agent for the tubercle bacillus was found to be present in the gamma-globulin fraction, especially from normal serum. Its bacteriostatic activity could be suppressed by the haptene tuberculopolysaccharide, indicating that this component may be the vulnerable site of the bacillus for attack.

The question arises whether this bacteriostatic agent may be lysozyme, which is known to occur in serum and cells, since it has an inhibitory effect *in vitro* on the growth of tubercle bacilli similar to gamma globulin. Moreover, it has been shown that one tuberculopolysaccharide (Type II) is rendered slightly more dialyzable in the presence of lysozyme, although it is not broken down to free glucose. On the other hand, another type of tuberculopolysaccharide (Type I) was rendered less dialyzable, perhaps due to the fact that precipitation occurred between it and the lysozyme.

The Relationship of Certain Factors to Necrosis in Guinea Pig Tuberculin Reactions

CUTTING B. FAVOUR, Palo Alto, Calif.

Guinea pigs were vaccinated subcutaneously with heat-killed tubercle bacilli suspended in 0.1 ml. of light mineral oil. Three groups of animals (A, B, and C) received doses of 1–10 mg. (dry weight) of organisms. Group A received two doses one week apart; Group B, three to four doses in one month; Group C, doses at irregular intervals for nine months or longer. PPD skin tests (5 γ) and cardiac punctures (2 ml.) for total serum proteins, electrophoretic studies, and hemagglutinin studies were done one to three times a month in Group A; and at wider intervals in B and C. Weekly weights were recorded. Animals which failed to gain were vaccinated less frequently.

Average weights of 1,000 gm. or more were attained by normal and sensitized animals which at no time showed more than 1–2 mm. of necrosis at forty-eight hours in PPD skin tests. Failure to grow, or weight loss, was associated with: (1) necrosis of 4 mm. or more in diameter at some time in the course of vaccination and (2) periods of hypergammaglobulinemia. Maximum hypersensitivity tended to occur in the first few months of vaccination. Subsequent tuberculin anergy was seen in some animals with continued hypergammaglobulinemia and weight suppression.

Boyden and Middlebrook hemagglutinin titers tended to rise and fall together, and to be higher when there was necrosis in skin tests. Individual readings correlated poorly with all factors except the presence or absence of hypersensitivity.

It is concluded that vaccinated guinea pigs may exhibit hypergammaglobulinemia with or without hypersensitivity. Animals developing necrosis of greater than 4 mm. in diameter in skin tests exhibited a greater growth suppression and hemagglutinin response than did animals which had less intense, and less prolonged, delayed type of allergy to tuberculin.

These findings are indirect evidence that necrosis of tuberculin allergy in part may be an Arthus-type reaction, dependent on humoral factors, triggered off by a less intense delayed type of allergy dependent on cellular factors.

Globulin Fractions as Related to Immunity

IRENE G. MELVIN and H. M. VANDIVIERE, Chapel Hill, N. C.

Host resistance to tubercle bacilli involves a variety of defense mechanisms, indices of which are constantly being sought in experimental animals. Paper electrophoresis offers a partial avenue of approach because serum of rabbits infected with various strains of Mycobacteria yields particular and specific responses in the electrophoretic pattern.

Patterns of serum from normal rabbits have demonstrated the globulin fractions to be alpha 2, alpha 2 prime, beta, and gamma. Rabbits with progressive tuberculosis also presented a fraction with a mobility slightly less than the beta fraction, which was termed beta prime.

Rabbits responded with increases in beta and gamma globulin to initial vaccinations with three strains of BCG, R1Rv, and H37Rv. Booster injections, given after these fractions had returned to the normal base lines established previously for each rabbit, produced even more marked increases in these two fractions. If the booster doses were given while the gamma fraction was still at a high level, there was a continued decrease in this fraction.

When the rabbits were challenged with standardized suspensions of Ravenel, the controls developed progressive pulmonary disease indicated by chest roentgenograms with steadily increasing beta and gamma globulin fractions over a period of several weeks. Shortly before death the gamma globulin dropped while the beta remained high.

Rabbits protected with good vaccines also showed that the beta and gamma globulin increased after challenge but, after a few weeks, the rabbits which kept their disease well controlled as indicated by chest roentgenograms had a decrease in the beta fraction while the gamma remained high.

Rabbits showing extensive disease and high levels of beta globulin, when treated with isoniazid, responded with decreases in the beta fraction and corresponding clearing of the lung fields.

Changes in globulin fractions and the trend of beta and gamma fractions after challenge suggest a predictable measurable interrelationship with immunity.

Immunogenic Considerations of Several Antituberculosis Vaccines[1]

H. Stuart Willis, H. M. Vandiviere, and Margaret R. Vandiviere,
Chapel Hill, N. C.

Several established strains of BCG and R1Rv have been studied to establish their relative immunogenic capacity.

Challenge studies in "protected subjects" must be the final criteria for "degree of protection." Thus, approximately 3,000 guinea pigs have been randomly vaccinated intracutaneously, each with 0.1 ml. of homogenous suspensions of one of the several vaccines which were standardized for both potency and viability *before* use. Cord-formation titration, omental index, and formazan-curve interpolation were used as methods of quantitative standardization, and results were confirmed by long-range animal studies and colony counts.

Periodically the experimental and control animals (same environment) were tuberculin tested and, finally, quantitatively challenged with H37Rv. Volumes of allergic response[2] and duration of allergy have been determined for each group.

Animals have been challenged at intervals varying from six to thirty months following vaccination. Differences in the protection afforded by the various strains are negligible in the short-term vaccinated groups. Those challenged at intervals from twelve to thirty months present statistically significant differences for the several strains which are quantitatively comparable to above-potency tests. The weakest of the BCG strains tested afford little more protection than that demonstrated by the unprotected controls. The degree of protection by R1Rv vaccine is significantly higher than that afforded with the best BCG strain, evaluation of disease being based on Feldman's Index. To supplement this, comprehensive pathologic evaluations have been made from coded gross and microscopic preparations.

The ratio of the volume of allergic response, the duration of allergy, and the degree of protection stand in agreement for each strain. The weakest strain gives the weakest response in each case, and the most potent strain demonstrates the greatest response, with the others between.

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[1] This study was made possible by a grant from the Committee on Medical Research of the American Trudeau Society, Medical Section of the National Tuberculosis Association, supplemented by assistance from the North Dakota Tuberculosis and Health Association.

[2] Volumes are measured with vernier caliphers and calculated as the volume of a segment of a sphere ($V = \frac{1}{6}h(h^2 + 3r^2)$).

3A Symposium on Sarcoidosis

Epidemiology

JOHN S. CHAPMAN, Dallas, Texas

Previous studies of the distribution of sarcoidosis in the United States have indicated that the disease has a very high prevalence in the southeastern portion of the country. According to VA statistics, sarcoidosis seems to be sixteen to eighteen times as frequent among Negroes as among whites. Other American figures show a definite but lesser difference. They also reveal equal distribution between the sexes and a maximal incidence in the third decade of life. Except in very few areas the disease has not been encountered in people less than twelve years of age.

Attention is drawn to the fact that according to present information sarcoidosis is almost unknown among Chinese and among American Indians. Very scant reports from Africa seem to indicate that the Negro in his native habitat in Central Africa does not exhibit the disease, while those in South Africa do so. World distribution seems to place nearly all reported cases in the temperate zones, but this may be artefactual.

Various efforts are under way to develop from a study of ecology of the region an explanation for prevalence of sarcoidosis in the southeastern United States. Up to the present no significant discovery has resulted; comparison of ecology of this area with that of European areas of high incidence is very much needed.

The Coexistence of Sarcoidosis with Other Pulmonary Infections

J. RICHARD JOHNSON, Madison, Wis.

Sarcoidosis has been reported in patients suffering from tuberculosis, histoplasmosis, cryptococcosis, coccidioidomycosis, and blastomycosis. The capacity of several of these diseases to simulate "sarcoid tubercles" causes the entire concept of coexisting diseases to become controversial, and many will contend that sarcoidosis, in these situations, is simply a response of the patient to his primary infection.

If the two diseases are separate entities, the coexistence may merely be coincidence; however, another recognized possibility is that sarcoidosis may produce susceptibility to these infections.

Evidence for this latter concept is reviewed, and five cases are presented in which sarcoidosis is believed to coexist with tuberculosis, histoplasmosis, or actinomycosis.

Case 1: A patient was discharged from a tuberculosis hospital when he was discovered to have sarcoidosis, not tuberculosis. Within two months he developed fulminating tuberculosis with multiple osseous, pulmonary, and miliary lesions. Tuberculin tests remained negative before, during, and after recovery from the tuberculosis.

Case 2: A patient with tuberculosis converted his sputum under chemotherapy, but suffered increasing dyspnea and progressive pulmonary infiltrations typical of sarcoidosis. Node biopsies were negative, but tuberculin tests remained positive. Prednisone therapy was required to restore the patient to health.

Case 3: A patient with roentgenographic changes of sarcoidosis and splenomegaly for four years developed acute pulmonary histoplasmosis. Following initial response to prednisone and subsequent relapse, there was spontaneous recovery. Histoplasmosis C. F. titer was 1/256. Axillary-node and skin-lesion biopsies were interpreted as sarcoidosis, but *H. capsulatum* was later cultured from the axillary node. All histoplasmin skin tests have remained negative.

Case 4: A similar case of long-standing, biopsy-confirmed sarcoidosis with an intercurrent episode of histoplasmosis.

Case 5: A mink farmer became critically ill with pneumonia and empyema due to *Actinomyces bovis*. After subsidence of the acute illness, decortication was performed successfully. The spleen remained markedly enlarged, and the bilateral hilar and right paratracheal

adenopathy, poorly seen during the acute illness, emerged three months later in the typical picture of sarcoidosis. Scalene node biopsy revealed sarcoid tubercles.

Summary

Clinical features, serial roentgenograms, skin anergy, complement-fixation titers, and biopsies in the cases presented indicate the coexistence of sarcoidosis and a variety of infectious diseases. Although sarcoidosis may have predisposed to the acquisition of these infections, it has not prevented satisfactory recovery from them.

Diagnosis of Sarcoidosis

HAROLD L. ISRAEL, Philadelphia, Pa.

In an effort to reconcile conflicting reports on the specificity and reliability of the Kveim reaction, further tests have been made on 60 patients with sarcoidosis and 40 patients with pulmonary tuberculosis. The results differed notably from those obtained with the test material prepared two years earlier from lymph nodes excised from the same patient. In both studies, positive Kveim tests were found in approximately 25 per cent of the patients with sarcoidosis. No positive tests occurred in tuberculous patients in the current series, in contrast to the finding of positive tests with the initial material in 42.4 per cent of tuberculous subjects.

Variability of the test material appears to be the principal cause of these inconstant results, but variations in histologic interpretation also contribute to the difficulties encountered with this test; differences in the type of clinical material studied appear to be a minor factor. In its present form this test is not sufficiently reliable or reproducible to justify its use as a substitute for clinical and biopsy methods of diagnosis.

Prednisone and Prednisolone Therapy

LOUIS E. SILTZBACH, MARK M. IMBERMAN, and HOWARD GROSSMAN, New York, N. Y.

Thirty-seven patients with sarcoidosis have been treated with prednisone and prednisolone at the Sarcoidosis Clinic of The Mount Sinai Hospital, New York, in the last three years (1955–1958). This number represents about 30 per cent of all patients with sarcoidosis observed during this period. In our experience less than one-third of the patients with sarcoidosis require steroid therapy.

Therapeutic results were similar to those we obtained with cortisone in 33 patients treated during 1950–1955.

Use of prednisone and prednisolone was attended by ease of administration since salt-poor diets and potassium supplements were not necessary. Prednisone and prednisolone can be used interchangeably.

Mild side effects such as moon faces, hirsutism, insomnia, irritability, and gastric hyperacidity were encountered. There was no instance of diabetes, peptic ulcer, or hypertension. One patient with pulmonary sarcoidosis contracted tuberculosis while receiving prednisone. The tuberculosis readily responded to antituberculous drug therapy. Fifty-eight patients in all were treated with all forms of steroid therapy during 1950–1958, and this was the only instance of tuberculosis. A similar low incidence of tuberculosis was found in our series of sarcoidosis patients *not* treated with steroids: 3 of 150 patients. All patients with intrathoracic sarcoidosis now receiving prednisone or prednisolone therapy also receive 300 mg. of isoniazid daily. No fungal infection occurred among the 37 patients treated in this series.

Fresh lesions are more responsive than older ones. Even patients with late pulmonary fibrosis may occasionally obtain symptomatic relief with these agents.

Fever, weight loss, weakness, cough, dyspnea, blurred vision, hypercalcemia, hyperglobulinemia were suppressed or returned to normal levels.

As a rule, marked clearing occurred in lung lesions, peripheral lymph nodes, and ocular lesions. Less responsive were mediastinal lymph node enlargement and hepatosplenomegaly.

But when the nodes disappeared there was little tendency for them to recur. In contrast, cutaneous lesions were agreeably responsive to therapy, but they almost always recurred when the drugs were stopped.

No patients were cured by steroid therapy and there *was* a high relapse rate when the steroids were discontinued. The drugs' suppressive action, however, proved beneficial in selected instances in which the unremitting course of the disease was producing loss of organ function or was even life-threatening. More often, prednisone helped to rid a patient of symptoms causing significant disability, and many of the patients treated returned to normal daily activities while on a maintenance dosage of prednisone and prednisolone—usually 7.5–15 mg. Initial dosage: 20–30 mg. Average course: six months.

The present-day indications for steroid therapy in sarcoidosis are outlined.

3B Laboratory Research Including Tuberculosis

On the Role of the Thyroid in Native Resistance to Tuberculosis

MAX B. LURIE, PETER ZAPPASODI, RICHARD S. LEVY, and ROBERT G. BLAKER, Philadelphia, Pa.

In our studies on constitutional factors which may control native resistance of inbred rabbit strains to tuberculosis it was found that an increment of the BMR of 30 to 40 per cent induced by L-triiodothyronine suppressed, to a greater or lesser degree, the pulmonary tuberculosis produced by the quantitative inhalation of human tubercle bacilli in three races, CaC, AD, and III III C, of low and intermediate genetic resistance to the disease. The hormone also exerted a conspicuous suppressive influence on the development of an already existing tuberculosis when L-triiodothyronine was administered three weeks after the infection. The resistance of the most susceptible strain in our colony, C, could not be materially increased by this treatment. L-Thyroxine increased the resistance of another race of rabbits, III A, four times, compared with that of untreated litter-mate controls. It is noteworthy that the tuberculin sensitivity was markedly reduced in the experimental groups.

By cultural methods it was found that L-triiodothyronine markedly restrained the cumulation of the bacilli in the lungs and accelerated their transport to the draining lymph nodes. L-Thyroxine also reduced the cumulation of the bacilli in the lungs of race III III C. The inhibition of bacillary growth and the consequent reduction of antigenic stimulation probably account for the suppression of tuberculin sensitivity in the hyperthyroid rabbits, despite the tendency for an increment of their nonspecific inflammatory irritability and the hypertrophy of their lymphoid tissue. The difference in the host-parasite relationships between treated and untreated rabbits of the same genetic resistance was of the same nature as that between untreated natively resistant and susceptible rabbits.

Dinitrophenol failed to increase the resistance of rabbits to tuberculosis. It is evident, therefore, that the resistance-increasing influence of thyroid hormones is not due merely to their calorigenic action.

Hypothyroidism induced by propylthiouracil or thyroidectomy reduced resistance to tuberculosis of the highly resistant race III, of the race of low resistance CaC, and of the race of intermediate resistance, III A. Thyroidectomy and propylthiouracil reduced the nonspecific inflammatory irritability of the skin, increased the number of bacilli in the lesions, markedly extended their caseation, and thyroidectomy was associated with atrophy of the spleen. The tuberculin sensitivity was usually reduced. The effect of both procedures on the pathogenesis of tuberculosis simulated that of cortisone and altered the host parasite relationship in the direction characteristic of native susceptibility.

In preliminary observations it was found that antibody production against a nonspecific antigen, bovine serum albumin, can be markedly enhanced by thyroid hormone in AD rabbits, the resistance of which to tuberculosis can be increased by this hormone. The converse is also true. In the very susceptible race FC, neither resistance to tuberculosis nor antibody production can be increased by this means. These observations would suggest

that the thyroid hormone balance markedly affects the activity of the phagocytic or reticuloendothelial system.

Sonic Fragility of Leukocytes from Guinea Pigs Vaccinated with BCG

ROBERT A. PATNODE and PAUL C. HUDGINS, Washington, D. C.

In a recent publication by Fong and associates it was reported that monocytes from BCG-vaccinated rabbits resist the necrotizing effect of virulent tubercle bacilli *in vitro*. The effect was more marked with immune cells cultivated in immune serum than with similar cells maintained in normal serum.

The results of studies in our laboratory indicate that a somewhat similar situation prevails when monocytes are exposed to sonic vibration. In a preliminary experiment, cells of paraffin oil-induced peritoneal exudates from normal guinea pigs and from guinea pigs vaccinated with heat-killed tubercle bacilli (H37Rv) in paraffin oil were suspended in normal or immune serum and sonic treated (9 Kc, 75 v, 60 sec.). Total leukocyte counts were made before and after treatment. Immune monocytes suspended in immune serum were significantly more resistant to sonic disruption than similar cells in normal serum, whereas suspending normal cells in immune serum did not increase their resistance to sonic disruption. In the next study, 15 guinea pigs were inoculated intradermally with BCG (1 mg). After five, nine, twelve, sixteen, and nineteen days, monocytes were collected from 3 BCG-vaccinated and 3 control animals, suspended in homologous serum, and exposed to sonic vibration. Although the sonic fragility of control monocytes remained uniform throughout the experiment, immune monocytes collected twelve days after vaccination or later showed marked resistance to sonic disruption.

In addition, it has been found that, when thoroughly washed immune monocytes are suspended in normal guinea pig serum and incubated for six hours, there is released into the serum a factor capable of (*1*) protecting normal monocytes against sonic disruption and (*2*) rendering normal monocytes susceptible to lysis by PPD. A further study has shown, however, that susceptibility to cytolysis by PPD does not necessarily confer upon cells a resistance to sonic disruption. Normal monocytes were suspended in serum collected from BCG-vaccinated guinea pigs forty-eight hours after a tuberculin skin test. Although the cells were thereby rendered susceptible to cytolysis by PPD, they were no more resistant to sonic disruption than were cells suspended in normal serum.

Serum Concentrations and Urinary Excretion of Isoniazid, Streptomycin and PAS, and Their Metabolites, as They Affect Their Antituberculous Efficacy

ROBERT S. MITCHELL, DICK K. RIEMENSNIDER, JOHN R. HARSCH, and J. CARROLL BELL, Denver, Colo.

Isoniazid is altered metabolically in the human body to form derivatives which possess insignificant antituberculous activity. The pattern of this isoniazid alteration or "inactivation" usually does not change in an individual with the passage of time. Wide variations, however, are observed between different individuals.

Isoniazid inactivation patterns have been studied by measuring serum bioassay concentrations of free isoniazid at various times after a test dose. Experience with 294 persons examined routinely by this method during the past two years shows that about half of the patients are "slow" and half "rapid" inactivators of isoniazid. Lung tissue, especially when heavily involved with tuberculosis, and cerebrospinal fluid tend to reveal free isoniazid concentrations equal to or greater than those measured simultaneously in blood serum.

The speed and frequency of reversal of infectiousness in 106 patients with advanced cavitary pulmonary tuberculosis during the first twelve months of isoniazid, streptomycin, and para-aminosalicylic acid (PAS) treatment were slightly higher in patients with "slow" than in those with "rapid" inactivation of isoniazid, especially true in the early months of treatment. These patients were divided into those receiving 3 to 5 mg. of isoniazid per mg.

per day and twice-weekly streptomycin (conventional dosage) and those receiving 12 or more mg. of isoniazid per kg. per day and daily streptomycin for at least two or three months (high dosage). The difference between "slow" and "rapid" inactivators virtually disappeared in the high-dosage group and became greater in those receiving the conventional dosage.

The chlorodinitrobenzene test (Johnson) has been adopted to measure free isoniazid in serum after a 15 mg. per kg. test dose. The classification of patients into "slow" and "rapid" inactivators by this test has correlated well with the serum isoniazid bioassay test using a 4 mg. per kg. test dose.

The measurement of PAS and its metabolites in serum and urine has not been as well solved as has been the case with isoniazid. We have no proof as yet, however, that individuals with "rapid" inactivation of PAS are the same individuals who manifest "rapid" isoniazid inactivation and vice versa. In addition, we suspect but have no conclusive proof, that variations in the handling of PAS coincide with differences in therapeutic response to PAS.

Streptomycin serum bioassay concentrations do not vary from individual to individual as frequently nor to the same degree as is the case with isoniazid; furthermore, streptomycin concentrations seem to vary with the adequacy of renal function.

Pathogenicity and Cord Formation Titration[1]

H. M. Vandiviere, Irene G. Melvin, and H. E. Kennedy, Chapel Hill, N. C.

Rough, eugonic colonies of mammalian tubercle bacilli manifest characteristic growth known as "cord-formation" which has become the basis of a reliable *in vitro* test for determining the degree of potency of various strains of avirulent, attenuated, and virulent Mycobacteria.

This test is actually a titration of the capacity for cord-formation of various strains grown in liquid medium containing 0.5 per cent bovine albumin and 5 per cent human plasma with varying concentrations of Tween®. Cord-formation is determined by microscopic examination of smears made from cultures grown in approximately 17 different concentrations of Tween 80 (from 0.00 per cent to 0.15 per cent) at seven and fourteen days. The end-point or titer of cord-formation is the highest concentration of Tween 80 at which cording is discernible when smears are examined under low power, with the next highest concentration showing marked dispersion of cords.

Avirulent laboratory strains of Mycobacteria fail to form cords even in the absence of Tween 80, while the virulent (H37Rv, "typical wild," and Ravenel) maintain cords in concentrations of 0.1 per cent and above. The attenuated strains (as the various strains of BCG and R1Rv) maintain cords in varying but strain-specific concentrations of Tween 80, the more potent strains yielding higher titers than those of lesser potency. *In vivo* tests support the relationship of cord-formation titer to degree of potency of these strains.

There is consistency in the cord-formation titers for the given strains unless the method of culture propagability in the laboratory is such as to disturb the initial potency of the strains. Alterations in potency have occurred under certain laboratory conditions, and such changes have been verified by omental index, duration of allergy, volume of allergic response, and the status of disease.

Typical "wild" strains of tubercle bacilli isolated from sputum yield titers above 0.1 per cent Tween.

Cord-formation titers of "drug-mutated" and/or "atypical" Mycobacteria isolated from patients have been determined and these correlated with catalase and animal studies. But "smooth" strains do not produce a titer equivalent to other testing methods.

[1] This study was made possible by a grant from the Committee on Medical Research of the American Trudeau Society, medical section of the National Tuberculosis Association, supplemented by assistance from the North Dakota Tuberculosis and Health Association.

The Pathogenicity of Isoniazid-resistant, Catalase-negative Tubercle Bacilli for the Silicotic Host (Guinea Pig)

WILLIAM STEENKEN, JR., Saranac Lake, N. Y.; JAMES W. RALEIGH and D. H. CHAPMAN, Sunmount, N. Y.; and M. M. SMITH, Saranac Lake, N. Y.

In previous studies conducted by L. U. Gardner at the Saranac Laboratory, it was established that the attenuated strain of tubercle bacilli, R1Rv, would not produce progressive disease in normal guinea pigs when administered subcutaneously or by inhalation. However, when given by either route to guinea pigs that had well-established disseminated pulmonary silicosis, the animals would succumb to widespread pulmonary silicotuberculosis. Tissue removed from the animals at death containing large numbers of tubercle bacilli again would not produce progressive disease when concentrated and subinoculated into normal guinea pigs.

It is a well-substantiated fact that tubercle bacilli that are made highly resistant *in vitro* or *in vivo* to isoniazid and that have lost their catalase activity will not, as a rule, produce progressive disease when inoculated subcutaneously or when given by inhalation to normal guinea pigs. This important observation has been the subject of much speculation relative to the ability of such organisms to cause contact tuberculosis. In fact, claims have been made that such modified organisms are harmless for those who harbor them, as well as for healthy nontuberculous individuals who might come in contact with them.

Studies were conducted using the silicotic guinea pig to determine whether or not isoniazid-resistant, catalase-negative tubercle bacilli are capable of producing progressive disease in the altered host. Accordingly, 12 different strains of tubercle bacilli—10 human and 2 bovine—which were resistant to 25 γ or more of isoniazid, catalase-negative, and which had lost their ability to produce progressive disease in normal guinea pigs when given subcutaneously or by inhalation, were used to infect guinea pigs that had well-established silicosis.

Each of the 10 strains produced fulminating silicotuberculosis in the silicotic host, regardless of whether the organisms were administered subcutaneously or by inhalation. None of the normal controls developed disease.

These findings prove conclusively that isoniazid-resistant, catalase-negative tubercle bacilli are capable of producing progressive disease in guinea pigs that have well-established pulmonary silicosis. The assumption can also be made that such altered organisms may be harmful to humans who already have pulmonary silicosis and that isoniazid would not be the drug of choice in treating such an infection.

Pleural Fluid Amylase in Pancreatitis and Other Diseases

JAMES F. HAMMARSTEN, BARNEY LIMES, and WALTER L. HONSKA, JR., Oklahoma City, Okla.

Pleural effusions may occur as a complication of pancreatitis. Four patients were observed with this complication. The mean serum amylase in this group was 367 units and the mean pleural fluid amylase was 14,334. One patient had a pleural fluid amylase of 53,000 units. In all instances, the pleural fluid amylase was higher than the serum amylase. Serial observations in 2 patients showed a slower decline to normal of the pleural fluid amylase than of the serum values.

Twelve patients with effusions due to other diseases were studied. In this group, the mean pleural fluid amylase was 81 $\pm$ 40 units and the mean serum amylase, 158 $\pm$ 89. In all instances in which the pleural fluid and serum sample were obtained on the same day, the pleural fluid value was lower than the serum level.

A search for pleural fluid and the determination of the amylase content are useful diagnostic procedures in patients suspected of having pancreatitis.

Temporary Lung Density Increases in Immunized Mice Resembling Delayed Hypersensitivity Reactions

ALFRED J. CROWLE, Denver, Colo.

Delayed hypersensitivity traditionally has been very difficult to demonstrate in mice by techniques which succeed in other animal species and, consequently, many investigators have supposed that it does not exist. However, recent investigations in several laboratories using special methods strongly suggest that this kind of allergy can be elicited in mice.

While studying the progress of infection in normal and in H37Ra-immunized mice by the lung density technique, we have noted that between two and five days after intravenous infection with virulent tubercle bacilli a temporary increase in lung density appears in the immunized mice which is absent in the controls and which apparently is not due to the infection itself.

Further investigation of this temporary rise in lung density in immunized animals implies that it may be a manifestation of tuberculin allergy. It cannot be confused with classic immediate hypersensitivity, for mice strongly sensitized to bovine gamma globulin and then challenged and examined at autopsy thirty minutes later during severe anaphylactoid reaction showed no lung density increase (mean lung densities of 0.67 and 0.71 for test and control groups, respectively). These and currently accumulating observations are discussed with regard to whether the observed phenomenon truly is a delayed hypersensitivity reaction.

Biochemistry of the Lung: Some Aspects of Glucose Metabolism in Normal Lung

VERNE D. HOSPELHORN and MARTIN J. FITZPATRICK, Kansas City, Kan.

This report is concerned with the initial studies made as part of a comprehensive investigation of the basic metabolic properties of lung tissue. At present the pathways of glucose degradation in the lung are being studied.

In a number of animal tissues there are two routes for the degradation of glucose after conversion to glucose-6-phosphate: the Embden-Meyerhof glycolytic sequence leading to the production of pyruvic acid and the direct oxidative pathway leading to the production of pentose carbohydrates. This preliminary report deals with the determination of the presence and the activity in the lung of some of the enzymes participating in these metabolic processes. Activities of the enzymes, pyruvic kinase and lactic dehydrogenase (in the glycolytic sequence) in addition to glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase (in the oxidative pathway), have been determined in homogenates of lung tissue. Sources of tissue have been the guinea pig and operative specimens of the human lung.

Normal lung tissues of the human and of the guinea pig contain very active pyruvic kinase (PK), lactic dehydrogenase (LD), glucose-6-phosphate dehydrogenase (G6PD) and 6-phosphogluconate dehydrogenase (6PGD). Representative activities of these enzymes found in the lung are: Guinea Pig—PK-2800; LD-600; G6PD-90; 6PGD-60. Human—PK-2800; LD-250; G6PD-60; 6PGD-29. Activity is given as micromoles of substrate utilized/gram wet weight/hour.

These findings and additional information concerning the metabolism of glucose by lung tissue are discussed.

4A Symposium on Fungus Diseases

Histoplasmosis: Early Pathogenesis

JOHN J. PROCKNOW, MALCOLM I. PAGE, and CLAYTON G. LOOSLI, Chicago, Ill.

The natural route of infection with the organism *Histoplasma capsulatum* is generally considered to be via the respiratory tract. The chlamydospore of the mycelial phase is

inhaled, resulting in a primary pulmonary infection. Dissemination of the organisms throughout the reticuloendothelial system occurs following conversion of the spore to the invasive yeast phase.

This study was planned to demonstrate the initial histologic reaction to the histoplasma spore within the mouse lung during which the conversion of the spore to the yeast phase takes place. The natural mode of infection was simulated by introducing a saline suspension of mature tuberculate chlamydospores into the nares of ether-anesthetized white female mice. Approximately 700 15-gram mice were each infected with 5,000 to 20,000 spores. Plating techniques proved 60 to 80 per cent viability of the spores. Groups of 3 to 6 mice were sacrificed at frequent (three-hour) intervals over a ten-day period for pathologic and cultural study. Others were observed for a more extended period (fifty-six weeks) and were sacrificed at two-day intervals. Lungs were fixed by the intratracheal injection of Zenkers-formol solution. Hematoxylin-eosin, hematoxylin-eosin-azure, Hotchkiss-McManus, and Gridley staining techniques were employed. Organ cultures on routine media showed that by three days dissemination had occurred.

Microscopic lung sections observed in sequential pattern beautifully illustrated the early tissue reaction to the spore and its disintegration. Mature typical tuberculate chlamydospores approximately 10 micra in size were seen in many alveoli. Within six hours they were "rimmed" by polymorphonuclear leukocytes. By eighteen hours alveoli containing spores had become clogged with polymorphonuclear leukocytes and epithelioid cells. Adjacent alveoli contained a few free macrophages. Exudate had spread into contiguous alveoli by thirty-six hours. At forty-eight hours spore tubercles protruded prominently and an internal trabeculation and segmentation occurred. By seventy-two hours some spores had ruptured and portions of the chitenous wall were phagocytosed by macrophages. At this time encapsulated yeast cells also were seen in free macrophages. Only a few spores persisted beyond five days and showed variable modes of disintegration. The widespread distribution of the progressive granulomatous infiltrates in proximity to the bronchioles was notable. Foci of infection in other organs indicated by small granulomas could be observed by six days.

Histoplasmosis: Course and Treatment

HARRY RUBIN, Kansas City, Kan.; J. LEWIS YATES and CHARLES A. BRASHER, Mount Vernon, Mo.; HOWARD W. LARSH and MICHAEL L. FURCOLOW, Kansas City, Kan.

This report concerns follow-up studies of 100 cases of histoplasmosis culturally proved in this laboratory. If time permits, discussion of the follow-up data of approximately 10 other cases of coccidioidomycosis and blastomycosis will be included.

At this time, follow-up observations have been obtained on 64 of the 100 patients with culturally proved histoplasmosis. The clinical classification and follow-up status of all of the cases are shown. These include 72 chronic pulmonary cases, 18 acute disseminated cases, 5 cases of acute pulmonary histoplasmosis, and 5 cases which we have not classified as yet.

Chronic pulmonary: Sixty-four of the 72 patients had cavitation demonstrable on their chest roentgenograms. Follow-up data have been obtained from 45 of these cases, and the length of follow-up varies from one month to twenty-six years from the time the first abnormal roentgenogram was taken. The average length of a follow-up study is forty-two months. Eleven of the 45 patients followed have died and all had extensive pulmonary disease. Four of the 11 died within ten days of major thoracic surgery from pulmonary infarction or surgical shock. Of the 45 cases followed to date, 5 patients have concomitant tuberculosis and 4 have bronchiectasis proved by tissue pathology or bronchograms.

Acute disseminated: A follow-up study has been completed on 15 of 18 patients in this category. Fourteen have died. The one known surviving patient was treated with amphotericin B and is well one year after treatment. Three of the 14 patients with fatal disease had clinical Addison's disease with Addisonian crises during life. At autopsy, 9 of the 14

showed involvement of the adrenals with *H. capsulatum*. The 3 patients with clinical Addison's disease had necrotic adrenals.

Acute pulmonary: Follow-up data have been obtained from only one of these 5 patients.

Data are analyzed from these 100 patients with respect to clinical and roentgenographic course, survival status, and their relation to skin test, serologic, and cultural data.

The Treatment of North American Blastomycosis: Treatment with Amphotericin B

E. Richard Harrell, Ann Arbor, Mich.

A total of 7 patients with a diagnosis of systemic North American blastomycosis has been treated by the intravenous administration of amphotericin B. All 7 patients evidenced pulmonary and cutaneous involvement and several had extensive bone involvement. Five of the 7 patients had been previously treated with large amounts of stilbamidine or 2-hydroxystilbamidine. These 5 patients had relapsed following the stilbene therapy. The blastomycin skin test was negative in all 7 patients, and the complement-fixing antibody titer was positive in 5 of them.

The response to amphotericin B therapy was very favorable in each instance. Six of the 7 patients are now free of evidence of active disease. The one treatment failure is a male with extensive pulmonary cavitation. The drug was administered intravenously in a dosage of approximately 1 mg. per kg. of body weight until a total of at least 1.0 gm. had been given. A febrile response, anorexia, and nausea accompanied even the slow administration of the drug.

Treatment of Coccidioidomycosis

David Salkin, San Fernando, Calif.

The disease caused by the fungus *Coccidioides immitis* was once confined to certain endemic areas in the southwestern portion of the United States. The organism is still confined to that area, but the tremendous movement of the population to and from that region has caused a dissemination of infected people, and the disease must be kept in mind in differential diagnosis in every state in the Union.

There is, as yet, no proved specific chemotherapy, although several new antimicrobials are under investigation, particularly amphotericin B. Current management is palliative and includes the proper use of rest, surgery, and supportive care. The evaluation of any specific therapy demands a knowledge of the natural history of the disease and the fate of acute infections, disseminated lesions, and residual lesions. The newly formed Veterans Administration-Armed Forces Cooperative Study may help to shed much light on the natural history of this disease and its treatment.

Cryptococcosis, an Increasing Problem

John S. Chapman, Dallas, Texas

Infection with *Cryptococcus neoformans*, in the past almost exclusively diagnosed in meningeal form, is being recognized more frequently prior to the development of meningitis. Present evidence seems to indicate that the disease is primarily an inhalational one, with implantation in the lung, followed by a low-grade pneumonia. Some clinical evidence exists that some of these lesions undergo gradual resolution and contraction. A nodule, indistinguishable from carcinoma or tuberculoma, may persist as a residue. In other instances the disease becomes disseminated and terminates as involvement of the central nervous system.

Tissue reaction is comparatively slight in most cases, and instances of meningitis are reported in which the mass of organisms acted more as an expanding lesion of the brain than as a meningeal irritant.

The behavior of the pulmonary infection leads to the suspicion that infection with the organism may be quite common, but that as in other mycotic diseases only rarely does this organism produce severe disease. The desirability of a skin antigen as well as serologic antigens is pointed out.

Cultural Characteristics of Systemic Fungi and Methods for Evaluating Therapeutic Agents

HOWARD W. LARSH, Norman, Okla.

The cultural and microscopic appearances of the various pulmonary mycoses are reviewed, stressing practical aspects. The use of the tissue culture technique for the conversion of these fungi from their saprophytic to the pathogenic phases is discussed. Recent development of, and the adaptation of the tissue culture method for, evaluating therapeutic agents have resulted in a more rapid evaluation and thorough testing of antifungal agents. Using the tissue culture method for the evaluation of antifungal agents, the present status of a number of new antimicrobials and chemicals for the control of systemic fungi are reported. In addition, the possibility of combined therapy is introduced.

If time permits, the increasing recognition of pulmonary blastomycosis and cryptococcosis in the midwest United States will be commented upon, as will the number of variants of *Histoplasma capsulatum* now under investigation.

Pathology of Fungus Diseases

HENRY C. SWEANY, Mount Vernon, Mo.

For a yet unexplained reason most deep fungal infections veer between a pyogenic inflammation and a granuloma. Sometimes one form predominates, sometimes the other, with most of the time a mixture of the two in varying proportions of each. In most instances there is no way of predicting what form will appear, but it must be some balance between the varying host and a multiplicity of antigenic substances arising from the particular parasite.

Some fungal infections may be diagnosed by the type of gross and microscopic lesions formed, but some are so protean in nature that it requires special culturing and staining as well as animal inoculation to decide the nature of the infecting agent.

In this brief report only a few of the common forms will be dealt with, leaving out such forms as monilia (candida), geotrichum, penicillium, aspergillus, mucor, and many more.

The main pathologic features of the following are discussed: actinomycosis; norcardiosis; blastomycosis, both North and South American; cryptococcosis; coccidioidomycosis; histoplasmosis; and sporotrichosis.

4B Atypical Mycobacteria

Purified Protein Derivatives (PPD) and Other Antigens from Atypical Acid-Fast Bacilli and Nocardia Asteroides

LEWIS F. AFFRONTI, Philadelphia, Pa.

Purified protein derivatives (PPD) and the residual fractions, designated as II fractions, were prepared according to the method of Seibert from four strains of atypical acid-fast bacilli and from one strain of *Nocardia asteroides*, using new glassware for each strain. These organisms included two strains from Kansas City (Bostrom D-35 and Forbes-84), two strains from Battey State Hospital, Rome, Georgia (No. 100616 and No. 121326), and a *Nocardia asteroides* (No. 2412) from Duke University.

Growth on Long's liquid medium was distinctive for each strain. All strains gave a characteristic orange, butyrous growth on Long's solid medium, but a yellow, soft growth on glycerol agar when initially planted on these two media. Growth of the *Nocardia asteroides* did not differ on these solid media.

The Kansas City strains showed similar microscopic morphology which differed from that of the two Battey strains.

Ultraviolet absorption studies of the PPDs revealed a maximal absorption at 270 mu to 275 mu. The ratios of the optical densities at the maxima over that at the minima gave a value similar to that obtained with PPD-S. The specific density values of Battey 121326 PPD and *Nocardia asteroides* (2412) PPD were high (3.17 and 3.48, respectively), indicating a high nucleic acid content. Although all of the PPDs contained desoxyribose nucleic acid, most of the nucleic acid in *Nocardia asteroides* (2412) PPD was apparently in the form of ribose nucleic acid.

The PPDs and the II fractions cross reacted with antisera prepared against each of the atypical strains and the BCG strain. Some protein specificity was shown with the PPDs since the highest titer usually occurred with the PPD and its homologous antisera when high dilutions were used in the precipitin test.

Agar gel studies not only demonstrated the antigenic complexity of these fractions, but also that these atypical strains are related to the human strain since cross reactions readily occurred.

Skin tests in sensitized animals showed both cross skin sensitization and specificity since the largest reactions were elicited by the homologous PPD.

Testing with Tuberculins Prepared from Atypical Acid-Fast Organisms

LYDIA B. EDWARDS and CARROLL E. PALMER, Washington, D. C.

To obtain a better understanding of low-grade, nonspecific (nontuberculous) tuberculin sensitivity which is highly prevalent in some parts of the country, a study is under way using a variety of tuberculins made from various acid-fast organisms. The response to two "atypical" tuberculins is compared with the response to PPD-S in tuberculous patients and healthy young adults.

The Correlation of the Reaction to Protein from Chromogenic Mycobacteria to the Reaction to Old Tuberculin

JOSEPH D. ARONSON, Philadelphia, Pa.; and WILLIAM KRAUS, Harrisburg, Pa.

The reaction to Old Tuberculin (OT) was compared with the reaction to proteins isolated by trichloracetic acid precipitation from a number of unheated and heated cultures of chromogenic mycobacteria. Tests were conducted on 1,210 patients in tuberculosis sanatoriums and on 1,322 male inmates of an industrial school. Each person was injected simultaneously with 0.00002 mg. of protein from one of the chromogenic mycobacteria and with 0.01 mg. of OT. If either reaction or both were negative or doubtful after forty-eight hours, 0.0002 mg. of protein and/or 0.1 mg. of OT was injected. Among patients in the sanatoriums, 91.6 per cent reacted to 0.01 mg. of OT and 6.1 per cent to 0.1 mg. of OT. The reactions to 0.00002 mg. of the proteins from the various mycobacteria ranged from zero to 86.0 per cent. In the industrial school population, 44.4 per cent and 11.0 per cent reacted to 0.01 and 0.1 mg. of OT, respectively. The reactions to 0.00002 mg. of the proteins ranged from 1.0 per cent to 70.7 per cent. The reactions to protein from unheated cultures differed in some instances from the reactions to protein of heated cultures. Among 420 males negative to OT and to protein from different mycobacteria before BCG vaccination, postvaccinal tests with 0.1 mg. of OT gave 92.4 per cent positive and 6.9 per cent doubtful reactions. The reaction to the proteins after BCG vaccination ranged from zero to 35.3 per cent with 0.00002 mg. of protein and from zero to 60 per cent with 0.0002 mg. of protein. The frequency and intensity of the reactions to the proteins from the various mycobacteria roughly paralleled the frequency and intensity of the reactions to OT. In general the reactions to the proteins were less marked and tended to fade more rapidly than the re-

action to OT. A higher percentage of Negroes than of whites reacted to the proteins. The percentage of nitrogen present in the protein was unrelated to skin sensitivity.

Clinical Evaluation of 100 Patients with Chronic Pulmonary Disease Due to or Associated with Atypical Mycobacteria

ALBERT G. LEWIS, JR. and FRANK P. DUNBAR, Tampa, Fla.; ROBERTS J. DAVIES, Tallahassee, Fla.; EUNICE M. LASCHE and ERNEST N. LERNER, Tampa, Fla.; DWIGHT J. WHARTON and JAMES O. BOND, Jacksonville, Fla.

During a 24-month period, 100 cases of patients with chronic pulmonary disease with atypical mycobacteria in their sputum have been collected in the Florida tuberculosis hospitals. These atypical bacilli vary in multiple characteristics from *M. tuberculosis*, and can be distinguished from each other. Photochromogens (*6*), nonphotochromogens (*76*), scotochromogens (*15*), and unclassified organisms (*3*) have been isolated. These atypical organisms have been subjected to animal virulence and a number of differential *in vitro* studies. Drug-susceptibility studies were done in all cases utilizing varying dilutions of isoniazid, PAS, streptomycin, viomycin, cycloserine, streptovaricin, and Promizole®. In 20 cases, drug-susceptibility studies were done on a number of other drugs.

A summary of laboratory methods and results and the current status of epidemiologic studies is presented. This paper is primarily concerned with clinical analysis of the cases—especially admission findings, course of illness, and evaluation of clinical roentgenographic findings before and after various drug regimens. Most of the cases had repeated and regular isolation of atypical acid-fast bacilli from their sputum over an extended period of time. Other causative organisms have been excluded in most cases.

Evidence is presented to support the conclusion that chronic pulmonary disease may be caused by a spectrum of atypical mycobacteria, which differ from *M. tuberculosis*. The nonphotochromogenic type was most commonly found, but photochromogens and scotochromogens were occasionally encountered.

As a group, the organisms are resistant to isoniazid and PAS and partially resistant to streptomycin. Susceptibility studies indicate the possible usefulness of other antimicrobials. Twenty-five patients had pulmonary resection. Medical and surgical treatment was highly effective in photochromogenic and scotochromogenic cases. There was a significant number of therapeutic failures in the group of nonphotochromogenic cases. Clinically, roentgenographically, and pathologically the cases are indistinguishable from tuberculosis. There is a striking predilection for white males more than forty years old.

The Production of Bacteriocin-like Substances by Strains of Atypical Mycobacteria

EMILIO C. MORA and A. EISENSTARK, Manhattan, Kan.

Filtrates prepared from broth cultures of four atypical strains of *M. tuberculosis* contained agents that inhibited the growth of a single specific strain of atypical *M. tuberculosis*. A fifth culture of Mycobacterium yielded a soluble product that inhibited the growth of a strain of *Mycobacterium phlei* and a strain of *M. tuberculosis*. Inhibition was demonstrated by placing a micro-drop of each filtrate on pour plates that had been seeded with the susceptible microorganisms. When sections of the clear areas produced by the micro-drops were added to fresh host cultures, no new lysis occurred. Inhibitory activity of the filtrates could be observed only at low dilutions, and no plaques were formed when these dilutions were plated. These findings were indicative of something other than phage activity. All five of the inhibitory substances were filterable through a Selas filter of 03 porosity. Preliminary studies indicated that pH changes were not responsible for the inhibition of growth; that the inhibitory substances were dialyzable and heat labile; and that the substances could be rendered inactive by protein denaturants such as acetone, ethanol, sulfosalicylic acid, and ammonium sulfate.

Quantitative Aspects of Neutral Red Reaction of Typical and "Atypical" Mycobacteria

LAWRENCE G. WAYNE, San Fernando, Calif.

The neutral red binding properties of mycobacteria have been related to their pathogenicity. Atypical mycobacteria, of lesser virulence for guinea pigs, show a reduction in intensity of neutral red reaction, as compared to virulent mammalian tubercle bacilli.

It is shown that the relative intensity of neutral red reaction is a function both of intensity of binding of the dye to the bacterial cells in alkaline suspension, and of the color of the dye that remains bound. Even saprophytes bind some dye, but the bound dye is yellow in alkali.

By prestaining suspensions of bacterial cells and treating them at various pH's, it is possible to quantitate the neutral red reaction as a function of time required for color change from red to yellow. A technique is presented which permits correlation of this quantitative neutral red reaction with other biologic properties of various inadequately defined mycobacteria.

4C Socioeconomic Problems in Tuberculosis Treatment

Out Patient Care of Tuberculosis

FRANCES S. LANSDOWN, JULIA M. JONES, and FANNIE BEHLEN, New York, N. Y.

There have been 621 patients treated for tuberculosis in the Out-Patient Clinic of Bellevue Hospital between April 1955–1957. The experience to be presented constitutes a final report of a demonstration and study at Bellevue Hospital supported by the New York Tuberculosis and Health Association. Final evaluation has been made of those cases in which treatment was completed by January 1, 1958.

A majority of patients began treatment in the hospital and, after careful evaluation of clinical, personal, and socioeconomic factors, were discharged to continue chemotherapy under clinic supervision.

In general, results of clinic treatment were good, but unfavorable results occurred in 26 per cent, as follows:

Clinical deterioration	4.4%
Progression of tuberculosis	1.2%
Complicating nontuberculous disease	3.2%
Drug intolerance	1.4%
Delinquency	18%

Delinquency was most frequent among patients with records of previous unsanctioned hospital discharge.

Infectivity of patients was a major concern. For this reason data were collected concerning infectivity during clinic treatment. In 42 patients tubercle bacilli were demonstrated in the sputum for periods longer than four weeks. The rest were either "sputum-negative" (culture) throughout their course of treatment outside the hospital, or were "sputum-positive" for only a brief period. New cases of tuberculosis were infrequent among persons living in contact with patients under treatment.

The experience indicates that treatment of new cases of tuberculosis is best begun in the hospital in order to facilitate complete clinical evaluation, make careful observation during early periods of therapy when drug reactions most frequently occur, minimize the dangers of infection of associates in the home, and teach the patient about his disease.

Social Attitudes of the Tuberculous Alcoholic and Their Bearing on Hospital Adjustment

KATE L. KOGAN, Seattle, Wash.

The question under investigation was whether psychological tests could make any practical contribution to the better understanding and handling of the alcoholic tubercu-

lous patient whose hospital management is a problem. At the termination of an exploratory study using a variety of psychological tests, it was concluded that most of the conventional clinical tools merely highlighted the variety of individual adjustment patterns and personality dynamics, and underlined the heterogeneity of persons who become alcoholic. One instrument, however, appeared to provide a consistent and measurable representation of the ways in which the hospitalized alcoholic tuberculous patient currently views himself and his social role.

A modified form of the LaForge and Suczek Interpersonal Checklist (ICL) was used. The ICL consists of 128 adjectives and phrases descriptive of interpersonal behavior. The patient was asked to classify each item on a nine-point scale of Social Desirability, to sort into two boxes labeled "Most People Are" and "Most People Are Not" and, finally to sort into two boxes marked "I am" and "I Am Not." Records were obtained from three groups of hospitalized tuberculous patients, 10 subjects in each group: Skid Row alcoholics, middle class alcoholics, and middle class nonalcoholics.

Analysis of the data revealed a series of scorable ratios which appeared to differentiate the pattern of attitudes of alcoholic subjects from the matched group who had no problem with alcohol. In summary description the alcoholic was deviant in his rigid attempts at conformity and being "super-normal." He intended to insist that he had the same kinds of qualities as most people and that he and everyone else met the ideal. It was possible to demonstrate that the strenuous efforts at appearing normal were a systematic defense in denial of inadequacy feelings. The relationship between the typical self-evaluation pattern and the occurrence of neurotic scores on the Cornell Medical Index (as obtained in another study) confirmed the impression that denial of difficulties was the essence of the defense employed.

Some of the implications of these findings for management are discussed.

The Tuberculous Alcoholic Before and During Hospitalization

EMILY B. FERGUS and JOAN K. JACKSON, Seattle, Wash.

"Skid Row" alcoholic males with tuberculosis are becoming a mounting problem to the agencies concerned with the control and treatment of tuberculosis and with rehabilitation. This paper explores some of the factors which contribute to the difficulties the patient experiences in following medical routines and in "adjusting" to the hospital and some of the ways in which the hospitalization encourages a return to the "Skid Row" and drinking following recovery from tuberculosis.

Comparison of the recent life experiences of alcoholics who became tuberculous and of alcoholics who did not would lead to the expectation that a larger proportion of tuberculous alcoholics were in a frame of mind conducive to recovery from alcoholism at the time of hospitalization. Almost half the tuberculous alcoholics and less than 20 per cent of the nontuberculous alcoholics had attempted a long period of sobriety during the year preceding the study. Yet 34 per cent of the nontuberculous alcoholics recovered from alcoholism, as compared with less than 5 per cent of the tuberculous alcoholics, and under conditions considerably less conducive to getting well.

Conditions in the sanatorium at the outset of treatment of tuberculosis were also favorable. The patient was emotionally vulnerable, accepted help from the staff, realized that his alcoholism had played an important role in the onset of his tuberculosis, and was totally removed from the associations and culture of the "Skid Row" which usually supported the persistence of his alcoholism. At the same time the demands of treatment for tuberculosis prohibited him from using most of the techniques which he had used in the past for rebuilding his defenses against help. At this stage he is usually regarded as a tuberculous patient and his alcoholism is considered a thing of the past or future.

His alcoholism becomes of concern to the staff when the patient has re-established his defenses, his "Skid Row" acquaintances, and thought patterns. This is the point at which help with his alcoholism is offered. By this time it is usually too late. His alcoholic ways of thinking and way of life have been re-established.

Other factors in his hospitalization experience which contribute to poor rehabilitation results on release from the sanatorium are discussed briefly.

Control of the Recalcitrant Tuberculous Patient

J. P. Myles Black, Olive View, Calif.

The Recalcitrant Patient Tuberculosis Unit (RPTU) in Los Angeles County is under the joint supervision of the Sheriff's Department, for custody, and Olive View Sanatorium, for medical supervision. The RPTU was initiated to maintain and treat: (*1*) patients in jail on Health and Safety Code violations and, (*2*) other misdemeanants in jail with diagnosis of active tuberculosis made on routine booking examination. All of the patients in the RPTU have been previously hospitalized at least once, unless the specific patient had refused hospitalization. More than 95 per cent of patients have previous convictions on other than Health and Safety Code violation.

Background information indicates that: 75 per cent of the patients are less than 50 years of age (this group is younger than the average sanatorium population); 15 per cent of the group are separated or widowed; the percentage of divorced, married, and single equally compose the remainder; the Caucasian group in the recalcitrant group is higher than in the normal sanatorium population with a smaller percentage of Mexican population and the same percentage of Negro in the recalcitrant as compared with the normal sanatorium population; 38 per cent of the patients had disease of longer than five years' duration, 26 per cent longer than two years' duration, and 35 per cent less than two years'.

In approximately 600 discharges from the RPTU, in a follow-up study of six to thirty-six months, we are unable to locate 29 per cent; 18 per cent are in jail at this time (15 per cent of this group or 3 per cent of the total group have inactive tuberculosis at this time); 15 per cent are currently hospitalized for treatment; 16 per cent are inactive, not working; 4 per cent have inactive disease and are working; 11 per cent are under adequate medical care; 6 per cent are dead, all due to progressive tuberculosis; only 0.5 per cent of these patients have been committed to mental hospitals with tuberculosis still active; 2 patients refused medical care but apparently are in good health; one patient refused to give any information concerning his progress.

Conclusion

These figures indicate that adequate tuberculosis control and some change in social behavior pattern have developed in those listed as having inactive tuberculosis and those currently hospitalized or under medical care (46 per cent). With this number of previously recalcitrant patients under good control, we feel that the maintenance of such a unit is mandatory in the tuberculosis control program in Los Angeles County.

5A Pulmonary Diseases in Children

A Report of 105 Patients with Cystic Fibrosis of the Pancreas Studied over a 5–14 Year Period

Harry Shwachman and Lucas L. Kulczycki, Boston, Mass.

A series of 105 patients with cystic fibrosis of the pancreas studied over a five- to fourteen-year period is presented. A brief outline of diagnostic criteria and the therapy employed is given. The patients are divided into seven groups and the division is based on the age at the time of diagnosis. Ten patients had meconium ileus, and were accordingly diagnosed within the first few days of life. Twenty-one patients were diagnosed when less than six months old. Fifteen patients were diagnosed between six months and one year; 18 patients were diagnosed between one and two years; 32 patients were diagnosed between two and eight years of age; and 9 patients between eight and sixteen years. At the present time 41 patients are more than 10 years of age. Ten of the 105 patients succumbed during the period January, 1956, to June 1, 1957, when this study was terminated.

A system of clinical evaluation is presented and is based on the following criteria: the general activity of the patient, the findings on physical examination, the nutritional status, and the findings on the roentgenogram of the chest. Each of these four items is given equal weight in a scoring system in which 100 represents a perfect score. It is believed that a scoring system to establish the degree of severity of cystic fibrosis is needed in view of the marked variation in the clinical manifestations of the disease.

The present condition of the 95 patients compared with their original status is as follows: 47 patients are now better; 27 are about the same; and 21 became worse. We should like to emphasize the value of pursuing a vigorous therapeutic program in a disease which is so little understood at present, with the hope that as a better understanding is achieved more effective therapeutic methods may become available. In our series of 105 patients 28 per cent were diagnosed after the age of three. It is hoped that the improved diagnostic tests now available will result in early diagnosis and prompt institution of effective therapeutic measures.

Adrenal Corticosteroids in the Treatment of Childhood Tuberculosis

MARGARET H. D. SMITH, JOHN C. CARDONA, NICHOLAS MATSANIOTIS, HORACE LICHTER, and SAMUEL STONE, New York, N. Y.

Since May, 1956, adrenal corticosteroids have always been used together with antituberculous drugs on the Tuberculosis Ward of the Children's Medical Service, Bellevue Hospital, in the treatment of 36 children with active tuberculous disease. Five patients were suffering from acute pleural effusion, their tuberculous etiology being proved by the presence of a positive culture or pleural biopsy in addition to a positive Mantoux reaction; in all cases clinical and roentgenographic clearing was unusually prompt. The long-standing pleural effusion in a sixth child appeared unaffected by the use of steroids. Three patients with tuberculous meningitis received steroids: 2 were treated early with excellent results, one was treated very late without apparent benefit. The course of 2 patients with miliary tuberculosis appeared unmodified. Twenty-five patients with mediastinal and endobronchial tuberculosis were followed by both roentgenogram and bronchoscopy. These patients fall into three groups. In a few cases the adenitis shrank rapidly, the endobronchial lesions cleared, and the involved lobe re-aerated. Many patients showed more rapid clearing of lesions than usual. A third group of patients (a small one) apparently did not improve with steroid therapy.

All of our patients received prednisone (Meticorten®) by mouth in a dose not exceeding 1 mg. per kg. of body weight daily, except for 2 patients treated with hydrocortisone. Most patients received treatment for about six weeks; in some cases a "rebound" phenomenon necessitated a second course of treatment. Untoward effects from the steroid were not seen, nor was there evidence of tuberculous spread. The greatest benefit was apparent in those patients treated early in the course of their disease.

In conclusion, adrenal corticoids can safely be used, together with antituberculous drugs, in the treatment of tuberculosis in children. They are likely to be beneficial in early cases of pleural effusion, meningitis, and endobronchial disease.

Experience with the Heaf Test in Children

MARGARET H. D. SMITH and MRS. SHIRLEY ANDERSEN, New York, N. Y.

A total of 820 children were tuberculin tested in the Pediatric Clinic of Bellevue Hospital. The Heaf test was performed on one forearm, on the other a Mantoux 5 TU; 706 patients (86 per cent) returned for reading, 70 (10 per cent) of whom were positive. Forty-eight of these were positive to both Heaf and Mantoux tests. Twenty-two others showed positive Heaf and negative Mantoux reactions. Seventeen of these 22 returned for testing with 100 TU and 250 TU Mantoux; the 4 showing negative Mantoux (5 TU) and *questionable* 1+

Heaf were negative on re-testing with 100 TU; of the 12 showing *definite* 1+ Heaf, 9 were positive to 100 TU, 2 were positive only to 250 TU, and one failed to react to either. The one patient with 2+ Heaf and negative Mantoux 5 TU showed a positive reaction to 100 TU. To compare the extent of induration and necrosis from Heaf and 5 TU Mantoux, respectively, 61 tuberculous children under treatment received both tests: the mean size of the Mantoux was 16 mm.; of the Heaf tests, 10 mm. Daily readings in these same children showed that Heaf, like Mantoux, tests must be read within five days.

In our experience the Heaf test was quick and easy to perform, picked up all reactors to the 5 TU Mantoux test, also some patients who reacted to 100 TU and 250 TU but not to 5 TU. Among positive reactors, the induration and necrosis produced by the Heaf test were less unpleasant than with the Mantoux.

The Application of Image Intensification and Cinefluorographic Technics to the Study of Chest Disease in Infancy

JOHN A. KIRKPATRICK, JR. and C. ROBERT E. WELLS, Philadelphia, Pa.

The fluoroscopic examination is potentially hazardous because of the amount of radiation utilized. There is no other method, however, that so readily permits the observation of physiologic phenomena. The image intensifier has made for more definitive fluoroscopic examinations at greatly reduced exposures to the patient and permits the recording of the fluoroscopic image photographically for subsequent study and teaching.

The nature of certain disease processes in early life is such that observation of physiologic phenomena is necessary for early and definitive diagnosis. Particularly are disease processes of the chest in the infant manifest by alterations in function. Acute inflammatory disease of the lungs in an infant may be first manifest by obstructive emphysema that may be difficult or impossible to recognize on roentgenographic study alone. Disturbances in aeration of the lungs accompany many congenital malformations of the tracheobronchial tree, the lung parenchyma, and the heart and great vessels.

An appreciation of the physiologic manifestations of disease is essential to the correlation of clinical, roentgenographic, and pathologic information leading to early diagnosis and treatment.

Cinefluorographic studies are projected to illustrate the place of this roentgenographic tool in the diagnosis of diseases of the chest in infants.

Diagnostic and Therapeutic Considerations Relative to Lobar Emphysema of Infancy

OSLER A. ABBOTT, WILLIAM E. VAN FLEIT, and HEINZ S. WEEMS, Emory University, Ga.

An initial experience wherein lobar emphysema of infancy was imitated by compensatory contralateral emphysema resultant to unilateral agenesis of the pulmonary artery initiated this study. A potentially catastrophic operation was avoided by angiography. The sites whereat distensive lobar emphysema of infancy may present, as well as the various causative factors involved, are reviewed. Two groups of patients, one with lobar emphysema of infancy and the other with unilateral agenesis of the pulmonary artery, have been analyzed in regard to findings on: (a) postero-anterior roentgenograms, (b) cinefluorographic study, and (c) angiocardiography. Considerations relative to the place of bronchography and bronchoscopy are included. The accepted therapy for "congenital" lobar emphysema consists of early pulmonary resection.

Review of this material makes us conclude that in patients with suspected lobar emphysema of infancy one must make: (a) analysis of the pulmonary vascular shadows on P-A roentgenograms, (b) fluoroscopy to define the ventilatory status of the suspected lobe, and (c) angiocardiography to define the extent of disease and rule out compensatory emphysema. Bronchography is of little value in definition or differential diagnosis. Bronchoscopy is considered essential. Lobar emphysema of infancy may be seen resultant to: (*1*) idiopathic cause or (*2*) congenital bronchostenosis.

A Rational Clinical Approach to Traumatic Thoracic Aortic Aneurysms

B. Eiseman and W. G. Rainer, Denver, Colo.

The increasing incidence of thoracic aortic aneurysms which follow nonpenetrating trauma has increased our experience sufficiently to allow some tentative conclusions on the natural history of this type of arterial injury. It would appear that such aneurysms in an otherwise normal aorta have a different prognosis than aneurysms which occur in an atherosclerotic or otherwise diseased vessel.

Six post-traumatic thoracic aortic aneurysms are presented which demonstrate essentially every phase of this condition, ranging from an asymptomatic aneurysm that has not altered in the forty-three years since injury to an individual who developed an aneurysm in the three weeks following injury while under observation.

Practical criteria for determining the advisability of resecting these aneurysms are presented on the basis of the known facts concerning the natural history of these lesions.

Coronary Arteriography

J. Stauffer Lehman, Philadelphia, Pa.

This report will describe the experience of the author in the performance of several procedures designed for the roentgenographic opacification study of the coronary arterial system.

The effectiveness of each of the several procedures in obtaining coronary artery visualization is discussed and technical details of each procedure are described.

Experiences with the clinical application of each of the several procedures are thoroughly discussed.

Relationship of Physical Activity to the Development of Coronary Atherosclerosis and Sudden Death from Coronary Occlusion

David M. Spain and Victoria A. Bradess, Brooklyn, N. Y.

Physical activity as related to coronary arterial disease consists of several distinct aspects. First, the relationship of activity or lack of it to the pathogenesis of the atherosclerotic process; second, the relationship of physical exertion to the precipitation of manifestations of acute coronary occlusion; and, third, the effect of activity on the function of the recently or remotely damaged myocardium.

Evidence will be presented based upon approximately 700 consecutive sudden deaths from coronary arterial disease which indicates that white males in sedentary occupations tended to die at a younger age than those engaged in more active occupations.

A related study will be presented based on several hundred consecutive necropsies on "normal" white males who died suddenly from accidents. This study shows that there is no significant difference in the amount of coronary atherosclerosis in various age groups as related to differences in occupational physical activity. These findings would indicate that differences in collateral circulation or the condition of the myocardium may be more important factors than the degree of coronary atheroma in the earlier deaths in the sedentary group as compared to the active group.

Investigations on the relationship of severe physical exertion to the precipitation of sudden death in persons with coronary arterial disease in both the atherosclerotic and thrombotic groups will also be presented. This latter study is based on more than 500 consecutive necropsies on white males who died suddenly from coronary arterial disease, and points toward the lack of any such significant relationship in the vast majority of cases.

Factors Affecting Intracellular Lipoid Deposition in Human Aortic Cells in Tissue Culture

David D. Rutstein, Estelle Fasolino Ingenito, John M. Craig, Marcello Martinelli, Mary Lou Foley, and Burton Goldberg, Boston, Mass.

A standard tissue culture of human aortic cells in a medium containing human serum was developed. In such cultures, intracellular lipoid deposition occurs after the addition of

cholesterol in a concentration of 1 to 5 mg. per 100 ml. in alcohol and 30 to 70 mg. per 100 ml. bound to beta-lipoprotein. The intracellular lipoid rapidly disappears when the cells are again exposed to normal medium. Intracellular lipoid deposition is completely inhibited by the simultaneous addition of 1 mg. per 100 ml. of linolenic acid and is potentiated by 1 mg. per 100 ml. of stearic acid.

These observations are being extended to other lipoid substances and other fatty acids. The technique is now being applied to the study of human atherosclerosis. The method is being supplemented by appropriate biochemical and tracer procedures in an attempt to elucidate the mechanism of the phenomenon.

6A Nontuberculous Diseases of the Lungs

Pulmonary Alveolar Proteinosis: A New Entity

SAMUEL H. ROSEN, Washington, D. C.; BENJAMIN CASTELMAN, Boston, Mass.; and AVERILL A. LIEBOW, New Haven, Conn.; FRANK M. ENZINGER, Washington, D. C.; and RICHARD THOMAS N. HUNT, Boston, Mass.

Twenty-six instances of a pulmonary disease resembling, but we believe different from, *Pneumocystis carinii* infection, have been encountered by the writers within the past five years. The process consists of filling of the alveoli by a PAS-positive proteinaceous material rich in lipids. This material appears to be produced by the lining cells which slough into the lumen, ultimately becoming necrotic and yielding granules and variable laminated cystlike bodies to the alveolar content. Reaction to this material is minimal.

Clinically, the disease may or may not be initiated by symptoms suggesting pneumonitis. The most common and prominent complaint is dyspnea, usually accompanied by a cough that may be productive of yellow sputum. Weight loss, fatigability, and chest pain occur in a small proportion of the patients. Cyanosis has been observed when the involvement is most severe. The lung fields are usually negative to percussion and auscultation. Roentgenographically, however, the appearance is that of a diffuse perihilar, radiating, feathery, or vaguely nodular soft density resembling that seen in severe pulmonary edema. The clue to the diagnosis is the inappropriateness of the symptomatology in view of this appearance.

The occupations of the patients have been various, the only clue to possible exposure to an injurious inhalant being that 4 of the 26 worked in lumber yards and 2 were electricians. Most of the patients were young adults, twenty to forty years of age, but there was one child of two and a half years.

The prognosis varies. Four patients have had a relatively stationary course, one for four and a half years without change in the roentgenograms. At least 2 are showing signs of recovery. At least 7 have died and, in 3 of these, fungi of diverse types have produced lesions superimposed upon those of the characteristic disease.

Because of present ignorance of the etiology of this condition and because of the characteristics of the material in the alveoli, we have tentatively designated it as "alveolar proteinosis."

Intrathoracic Complications of Subdiaphragmatic Infection

DAVID P. BOYD, Boston, Mass.

While this presentation will deal only with intrathoracic complications, the entire subject of infection of the subphrenic space should be of great interest to thoracic specialists.

A study of 115 cases of subphrenic infection seen at the Lahey Clinic in the past few years forms the basis for this report. A number of these cases showed interesting complications within the thorax.

The surgical and pathologic anatomy of the diaphragm and suprahepatic spaces has been a source of confusion to students in the past. An attempt has been made to simplify this anatomy and pathology on the basis of anatomic studies and clinical findings.

The chronicity of subdiaphragmatic abscess today may have resulted in an increased

frequency of intrathoracic complications. Examples will be cited in which the first symptom of infection below the diaphragm was the profuse expectoration of purulent material.

We have seen cases of bilateral subphrenic infection with bronchial fistula. These patients may not require pulmonary surgery if the subdiaphragmatic infection is adequately drained. The importance of adequate drainage is emphasized by reference to case reports of patients who have died of fulminating bronchopneumonia as a result of acute massive perforation of such an abscess through the diaphragm into the bronchial tree.

A most interesting group of cases (closely related to the above) are those of bronchobiliary fistulas. We have seen a number of patients with postoperative strictures of the bile ducts who have accumulated subphrenic collections of bile which in turn perforated into the free pleural cavity. These are cases of pleurobiliary fistula. Other patients with pleural fusion have developed bronchobiliary fistula. Examples in each category are shown.

Conclusions

Infection of the subdiaphragmatic space is confusing to both the student and practitioner with regard to its surgical anatomy, pathologic anatomy, and nomenclature. (*1*) An attempt is made to simplify these important subjects. This involves revision of our traditional concept of the anatomy of the suprahepatic space on both sides. (*2*) Intrathoracic complications of subdiaphragmatic infections are increasing because of the attenuation of these infections by modern methods of treatment. (*3*) A group of representative cases of intrathoracic complications of subphrenic infection are cited. These cases include acute fulminating perforation of subphrenic abscess through the diaphragm into the lungs; chronic perforation and fistula between these two areas; and chronic pleurobiliary and bronchobiliary fistulas.

Pulmonary Capillary Proliferation Induced by Oxygen Inhalation

PHILIP C. PRATT, Columbus, Ohio

Capillary proliferation in alveolar septa has been mentioned by many authors in the course of publications describing pathologic findings in a wide variety of diseases. The proliferation is usually considered to be a manifestation of the underlying disease, but the resultant appearance is so similar as to suggest a causative agent common to all of the diseases. Such an agent is oxygen which is almost always administered to patients in the terminal stages of progressive pulmonary insufficiency from whatever cause.

With this possibility in mind, a study has been conducted to ascertain whether capillary proliferation can be induced in human lungs by oxygen inhalation. Two groups of cases have been reviewed, one selected from consecutive clinical records of patients examined at autopsy after having received oxygen therapy, and the other a consecutive series of autopsies. The material reveals that, in each series, those patients who had oxygen exposure showed capillary proliferation. In most cases the primary disease for which oxygen was given was not pulmonary.

The implications of this observation with respect to the toxicity of oxygen and the pathology of various obscure pulmonary diseases are discussed.

Bronchogenic Carcinoma

Experimental Lung Cancer

MARVIN KUSCHNER, SIDNEY LASKIN, and NORTON NELSON, New York, N. Y.

The increasing incidence of lung cancer has led to the accumulation of much epidemiologic data on the relationship between this tumor and exposure to suspected environmental carcinogens. Experimental evaluation of suspected carcinogens is essential if one is to examine a variety of suspected materials and define the specific carcinogenic components of complex materials. Laboratory reproduction of bronchogenic carcinoma further permits a detailed study of stages in the pathogenesis of lung cancer.

Using such known carcinogens as the polycyclic hydrocarbons, methylcholanthrene, dibenzanthracene, and benzpyrene, the induction of tumors comparable to human lung cancers has been studied. Techniques of inhalation exposure, intratracheal instillation, thread transfixion of the lung, and intrabronchial pellet implantation have been used. Techniques so developed with known carcinogens have been applied to such suspect materials as tobacco tar fractions, compounds of chromium, and ionizing radiation.

As demonstrated by the results with ionizing radiation, such application may yield significant dosage data and contribute to an understanding of the stages of mucosal change leading to malignant transformation.

Tumor Cells in Bronchogenic Carcinoma

C. A. Ross, Buffalo, N. Y.

Patients with proved bronchogenic carcinoma have had studies of peripheral blood made for tumor cells. Approximately 15 per cent of all patients admitted with bronchogenic carcinoma have tumor cells in preparations made from peripheral venous blood. Studies have also been carried out, drawing samples from the pulmonary vein at the time of surgery both before and after manipulation. After manipulation, 70 per cent of specimens show tumor cells. A comparison of resectable versus nonresectable cases are made, showing that in nonresectable cases more than 50 per cent of specimens drawn from the pulmonary vein prior to manipulation showed tumor cells. Specimens drawn from a peripheral vein one week after resection have failed to show tumor cells in any case.

Newer Morphological Approaches to Prognosis in Lung Cancer

Fred C. Collier, J. F. A. McManus, Edmund A. Dowling, and Beulah M. Hathaway, Birmingham, Ala.; Robert H. Kyle, H. T. Enterline, and Stanley M. Becker, Philadelphia, Pa.

Recent presentations before this society emphasized the prognostic significance of vascular invasion in lung cancer. Statistical analysis of the data on which these presentations were based suggested that, unlike many other lethal carcinomas, when patients survived three years following resection for pulmonary carcinoma, five-year survivals were common.

Accordingly, additional cases were obtained through the Pulmonary Neoplasm Research Project and the Tumor Registry of the Alabama Association of Pathologists, yielding more than 300 patients whose primary lung tumors were resected sufficiently remotely to afford an opportunity for a three-year follow-up study. The recent study by Rosahn, comparing the results of surgically treated breast cancer managed by two different groups of surgeons, suggested the advisability of comparing the results of the different groups of thoracic surgeons who performed the resections reported in the current survey.

In the present group of patients, on whom resections were performed in many hospitals, the frequency of vascular invasion continued as a most striking phenomenon. Again, special histologic techniques—simple to perform, easy to interpret, and readily reproducible—enabled the investigators to determine the presence or absence of vascular invasion with great rapidity and accuracy. Independent agreement on the presence or absence of vascular invasion by two pathologists was marked in virtually all instances.

The correlation between the possibility of a surgical cure and the absence of histologically proved vascular invasion by tumor is exceedingly high when tested by chi square ($P = 0.001$). This observation invites solution of the problem of why a given lung cancer invades blood vessels when its structural, anatomic, and histologic counterpart does not. The results of the histochemical attacks helping to resolve this problem are shown, and the practical significance to the clinician of histochemical procedures hitherto regarded as abstruse are emphasized.

6B Problems Related to Chronic Lung Diseases

Chemoprophylaxis in Pulmonary Emphysema

ANNE L. DAVIS, EVELYN J. GROBOW, JOHN H. MCCLEMENT, and RALPH R. TOMPSETT, New York, N. Y.

This project was designed to study the effect of chemoprophylaxis on the bacterial flora of sputum and the incidence of respiratory infections in outpatients with clinical and physiologic evidence of pulmonary emphysema.

After at least three consecutive early morning deep cough specimens had been obtained, 29 patients with emphysema were placed alternately on tetracycline or tetracycline placebo for two days each week. Cultures were homogenized, plated on appropriate media, and studied in detail. All patients have been followed for more than twelve months with weekly or biweekly sputum cultures and obeerved clinically for evidence of lower respiratory tract infection, which was specifically defined.

In the pretreatment period the patients in the placebo group and those in the tetracycline-treated group were comparable in age, pulmonary function tests, sputum flora, and incidence of infection.

During the treatment period of nine to twelve months *Hemophilus* was isolated more than twice as frequently in the placebo patients as in the tetracycline-treated group. This organism could not definitely be correlated with clinical infection and was noted to come and go spontaneously in some patients without relation to treatment. Pneumococci were isolated sixteen times more frequently in the control patients than in the treated, and were often present without clinical infection. Gram-negative organisms were isolated in both the placebo and tetracycline-treated groups.

The incidence of respiratory infection in the placebo group was almost twice that in the tetracycline-treated patients. While this difference suggests a possible effect of tetracycline therapy, it was not considered statistically significant. The numbers in each group requiring other antimicrobial therapy or hospitalization were not significantly different.

There were no significant differences in the subjective reactions of the two groups, nor in the post-treatment pulmonary function tests.

In conclusion, tetracycline was effective in preventing the appearance of pneumococci and—to a lesser extent—*Hemophilus* in the sputum, but these effects could not be definitely correlated with the reduction in infections in the treated group. Moreover, the reduction in infections could not, with the available data, be attributed to tetracycline.

Kyphoscoliotic Cardiopulmonary Disease: A Reappraisal

GORDON L. SNIDER, GEORGE MILLER, BERNARD MILLER, and EDWARD I. ELISBERG, Chicago, Ill.

It has been common teaching for many years that severe kyphoscoliosis results in cardiac failure and death usually in the fourth or fifth decade of life. Twenty-six cases of severe skeletal deformities of the chest have been studied and the literature has been reviewed in an attempt to re-evaluate the association of cardiopulmonary disease with this condition. The patients ranged in age from six to seventy-one years; they were evaluated by means of history, physical examination, pulmonary function tests, roentgenographic examination, and electrocardiograms. Six patients underwent right-heart catheterization.

These cases were divided into two groups on the basis of a history of recurrent bronchopulmonary infection and rhonchi on physical examination. All patients demonstrated evidence of a severe restrictive ventilatory defect. Twelve patients in Group I without a history of recurrent respiratory infection had only this defect. With one exception these patients had no cardiorespiratory symptoms. One patient had dyspnea on effort due to an extremely severe restrictive ventilatory defect which was probably associated with alveolar hypoventilation. Fifteen patients in Group II gave a history of recurrent bronchitis and

had evidence of airway obstruction combined with their restrictive ventilatory defect. The 3 deaths in the series occurred among these latter patients, representing the only deaths from cardiopulmonary disease during the seven years in which this patient group has been collected and observed.

Two patients in Group I and 4 patients in Group II had cardiac catheterization performed. One patient in Group I and one patient in Group II had pulmonary hypertension and arterial oxygen unsaturation on exercise. All other patients had normal hemodynamics and arterial oxygen saturation. The patient in Group I with pulmonary hypertension was the one mentioned above with a very severe restrictive ventilatory defect and alveolar hypoventilation. The patient in Group II with pulmonary hypertension subsequently developed severe polycythemia and died of chronic cor pulmonale in intractable congestive heart failure.

Patients with severe kyphoscoliosis may thus usefully be classified into two groups: Group I without a history of recurrent respiratory infection, and Group II with such a history and evidence of bronchial obstruction on physical examination. Dyspnea on mild or moderate effort and the development of cor pulmonale are rare in Group I, although an occasional patient is seen with such a severe restrictive ventilatory defect that alveolar hypoventilation, hypoxia, pulmonary hypertension, and occasionally cor pulmonale may supervene. In such patients a lower respiratory tract infection may have catastrophic effects because of the very low pulmonary reserves. Dyspnea on effort, evidence of chronic airway obstruction, and abnormalities of blood gases are much commoner in patients of Group II than of Group I. The evidence suggests that alveolar hypoventilation occurs primarily due to abnormal distribution of inspired air which is primarily due to diffuse airway obstruction. Polycythemia, chronic cor pulmonale, and death are much commoner in these patients.

Generally speaking, the prognosis is not nearly as grave as the American and English literature has indicated in the past. Patients may not develop progressive pulmonary insufficiency. An understanding of the underlying pulmonary disease is the keynote in the treatment of pulmonary and cardiac failure complicating kyphoscoliosis.

Effects of Kyphoscoliosis upon Mechanics of Breathing in Children

COLIN G. CARO and THOMAS GUCKER, 3RD, Philadelphia, Pa.

The mechanics of breathing were studied in 5 children with poliomyelitic and 5 with nonparalytic kyphoscoliosis by measuring functional residual capacity and airway resistance (plethysmographic technique), lung compliance, alveolar gas uniformity, vital capacity, and maximal flow rates.

Control values were established in 13 to 18 normal children (age range six-fifteen years): functional residual capacity (L) = $-1.13 + 2.06$ [surface area (M^2)], airway conductance, panting $\left(\frac{\text{L/sec.}}{\text{cm}H_2O}\right) = -0.10 + 0.25$ [thoracic gas volume (L)], lung compliance ($\text{L/cm}H_2O$ = $0.006 + 0.056$ [functional residual capacity (L)], alveolar gas uniformity (percentage nitrogen increase) = 1.2 per cent (S.D. 0.3), maximal and expiratory flow rate (L/min) = $-51.6 + 197$ [surface area (M^2)].

In the kyphoscoliotics, functional residual capacity was normal or reduced. Hyperinflation was absent. Lung compliance was frequently reduced. Airway resistance was normal. The features of obstructive emphysema were not found. Uneven distribution of inspired gas and reduced maximal flow rates were commonly present and were thought to result from thoracic cage weakness or deformity.

The Place of Costo-Transversectomy in the Treatment of Scoliosis of the Thoracic Spine

LAMAR SOUTTER, Boston, Mass., and PAUL L. NORTON, Brookline, Mass.

Scoliosis consists of both lateral curvature and rotation of the spine. It is quite common, usually of little or no clinical importance, and it is idiopathic in most cases. It appears, as a

rule, in childhood when, except for its cosmetic effect, it causes no trouble. Patients with marked angulation of the thoracic spine begin to suffer from pulmonary insufficiency, cardiac failure, and severe arthritic pain in the third and fourth decades of life. In such persons the heart will lie in the concavity of the spinal curve and may be fixed or even compressed by its boney cage. One lung will be collapsed into a small area, the other overexpanded and deformed by rotation. With advancing age the chest wall gradually becomes fixed with premature calcification of rib articulations and ligaments; the diaphragm becomes ineffective because of the deformity. Dyspnea may be very severe. Death results from cardiac failure or intercurrent pulmonary infection, or both. No effective treatment has been possible in adult life, but considerable strides have been made by orthopedic surgeons in correcting the malleable curves in childhood, then fusing the spine to hold the correction. Plaster jackets have been useful tools for correction of curves in most instances.

In some cases the spine has become unyieldingly rigid at an early age, precluding more than slight correction prior to fusion. In such children with very marked deformity, we have employed costo-transversectomy as a means of obtaining greater mobility before applying a corrective plaster jacket. This operation consists of the resection of small portions of ribs and transverse processes within the concavity of the scoliotic curve. It is based upon the principle that after a thoracoplasty the spine will bend permanently away from the operated side. We have used it upon 10 children whose spines responded poorly to correction in a Risser plaster jacket. The results have varied considerably; in several cases the improvement did not justify the additional surgical procedure, but in others there was a sufficient change of the curve to be encouraging. In 2 cases the measure of improvement was more than 30 degrees. Because this experience has been spread out over a number of years, we have learned a good deal about the performance of the operation, the timing of the application of the jacket and of the spinal fusion. In our last case we have tried a different type of fusion by placing a bone graft into an intervertebral groove on the convexity of the interthoracic spine, hoping to secure better fixation and perhaps further correction by interference with the growth rate of the vertebral bodies upon that side. In conclusion, we have found that the operation of costo-transversectomy offers children with severe scoliosis and a prematurely fixed deformity of the thoracic spine an increased hope of correction of their curvatures so that they can enter early middle age with less threat of pulmonary insufficiency and cardiac failure than would otherwise be their lot.

Farmer's Lung: Clinical Physiology and Prognosis

QUINTON C. CALLIES, HELEN A. DICKIE, and JOHN RANKIN, Madison, Wis.

We have previously described an acute granulomatous interstitial pneumonitis which occurs among some agricultural workers exposed to unusual quantities of moldy forage. It has been shown that this is an easily recognized distinct syndrome with uniform clinical, roentgenographic, and pathologic features. The physiologic abnormalities demonstrated in 35 patients show a similar degree of uniformity. In most instances there was a considerable reduction in pulmonary diffusing capacity and gross abnormality in the distribution of inspired gas. These abnormalities were associated with but moderate hyperinflation of the lungs and mechanical abnormalities suggesting slight bronchial obstruction.

Although, clincally, recovery from any single episode was usually complete within a few weeks or months, it seemed to us important to determine whether residual damage likely to lead to chronic pulmonary insufficiency remained, and whether continued employment in agriculture was hazardous. Consequently, we have performed extensive pulmonary function studies on 24 farmers who had suffered from a typical attack one to eight years previously.

The results may be conveniently summarized as follows:

Group I: Those who had stopped farming altogether: In this group the residual abnormalities were minimal or insignificant.

Group II: Those who continued to farm but carefully avoided exposure to unusual quanti-

ties of mold-contaminated dusts: With few exceptions, disability was slight although physiologic abnormalities persisted.

Group III: Those who, for a variety of reasons, had been unable to avoid moldy dusts on occasion: Without exception, significant pulmonary insufficiency could be demonstrated, and in many there was incapacitating disability. The most constant physiologic abnormalities were a decrease in pulmonary diffusing capacity and gross abnormality in the distribution of inspired gas.

In conclusion, it would appear that, although the prognosis is favorable after any single attack, continued exposure (at times minimal) to moldy farm dusts is hazardous and may result in crippling chronic pulmonary insufficiency.

The Detection of Hepatic Toxicity Due to Pyrazinamide by Means of the Determination of Serum Enzymes

JOHN F. MORRISSEY and RUTH C. RUBIN, Madison, Wis.

During the past eighteen months we have studied 65 patients treated with a daily dose of 3.0 gm. of pyrazinamide by means of the serial determination of serum glutamic oxalacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), serum lactic dehydrogenase (SLDH), serum malic dehydrogenase (SMDH), bromsulphthalein retention, cephalin flocculation, and prothrombin time.

Elevations of SGOT occurred in 11 cases which required stoppage of the drug. When their serum enzyme concentrations had returned to normal, test doses of pyrazinamide were given to 6 of these patients. Three patients showed no reaction and have tolerated continued therapy. Three patients reacted with a moderate to marked rise in enzyme concentrations confirming the presence of drug hypersensitivity, and therapy was permanently interrupted. There was not sufficient clinical indication for the use of pyrazinamide to justify the risk of further therapy in the remaining 5 patients. Needle liver biopsy was performed in 4 of the above patients and only very minor histologic abnormalities were noted.

The SGOT proved to be equal to or more sensitive than any or all of the other enzyme tests. The SGPT was less frequently elevated; but associated elevations occurred in all the serious reactions. The SLDH and SMDH were found to be of much less value and were more often nonspecifically elevated. The bromsulphthalein was elevated above 15 per cent in only 2 cases in association with rises in enzyme concentrations.

A review of the literature revealed that 4.4 per cent of 801 patients treated with pyrazinamide and followed by the conventional liver function tests developed jaundice. The absence of jaundice in our study establishes the superiority of the SGOT over the conventional liver function tests in this situation with statistical significance at the 0.05 level of probability using the technique of sequential analysis.

NOTICES

The following dates and locations of the next two annual meetings of the National Tuberculosis Association and the American Trudeau Society have been selected and approved:

1959—Chicago, Illinois—May 24–29
1960—Los Angeles, California—May 15–20

ANNUAL MEETING OF THE AMERICAN TRUDEAU SOCIETY

The Medical Sessions Committee *INVITES SUBMISSION OF PAPERS* on all scientific aspects of tuberculosis and nontuberculous respiratory and cardiopulmonary diseases for presentation at the *1959 annual meeting in Chicago, Illinois, May 25–27.*

Abstracts

1. Abstracts of papers to be offered for presentation should be submitted before January 7, 1959, to the Chairman of the Medical Sessions Committee. *Seven copies* are required for distribution to committee members.

2. Abstracts should be limited to a 300-word summary including important data *and conclusions.* Charts and tables should be attached to aid in the selection of papers. Abstracts selected for presentation at the annual meeting will be published in THE AMERICAN REVIEW OF TUBERCULOSIS AND PULMONARY DISEASES.

3. The names, academic degrees, titles, and complete addresses of the senior author and co-authors should be included in each abstract.

4. All abstracts will be reviewed by the Medical Sessions Committee. Selection will be largely based on the areas of interest suggested by you and others receiving this invitation.

5. Those wishing to present subjects of a confidential nature from the armed forces or other sources should first clear the material through official channels.

6. Authors will be notified by March 1, 1959, if their papers have been accepted for presentation.

7. Papers presented at the annual meeting must be ORIGINAL contributions NOT PREVIOUSLY PRESENTED OR PUBLISHED. Medical papers presented at the meeting may be submitted to THE AMERICAN REVIEW OF TUBERCULOSIS AND PULMONARY DISEASES by the author if he so desires.

Suggestions

The Medical Sessions Committee is particularly anxious to build the 1959 program around your special interests. A memorandum suggesting subjects and speakers would be welcome and should be sent to the Chairman by October 15, 1958.

H. WILLIAM HARRIS, M.D. *Chairman*
Medical Sessions Committee
American Trudeau Society
1790 Broadway, New York 19, N. Y.

SCHEDULE OF LABORATORY REFRESHER TRAINING COURSES
July, 1958—June, 1959

The following schedule of laboratory refresher training courses will be offered by the Laboratory Branch of the Communicable Disease Center, Chamblee, Georgia.

Information and application forms should be requested from Laboratory Training

Services, Communicable Disease Center, U. S. Public Health Service, P. O. Box 185, Chamblee, Georgia.

Dates	Course Number	Courses	Duration	Location*
			weeks	
Sept. 15–Oct. 10	8.00	Laboratory Methods in the Diagnosis of Parasitic Diseases		
		Part 1. Intestinal Parasites	4	1
Oct. 6–10	8.26	Laboratory Methods in the Diagnosis of Rabies	1	2
Oct. 13–24	8.20	Laboratory Methods in the Diagnosis of Viral and Rickettsial Diseases	2	3
Oct. 13–31	8.01	Laboratory Methods in the Diagnosis of Parasitic Diseases		
		Part 2. Blood Parasites	3	1
Oct. 20–31	8.55	Laboratory Methods in the Diagnosis of Tuberculosis	2	2
Jan. 5–30	8.15	Laboratory Methods in Medical Mycology Cutaneous, Subcutaneous and Systemic Fungi	4	1
Jan. 12–16	8.26	Laboratory Methods in the Diagnosis of Rabies	1	2
Jan. 26–Feb. 6	8.55	Laboratory Methods in the Diagnosis of Tuberculosis	2	2
Feb. 9–20	8.17	Laboratory Methods in the Study of Pulmonary Mycoses	2	1
Mar. 2–6	9.40	Laboratory Diagnostic Methods in Veterinary Mycology	1	1
Mar. 2–13	8.40	Laboratory Methods in the Diagnosis of Bacterial Diseases		
		Part 1. General Bacteriology	2	2
Mar. 9–20	8.20	Laboratory Methods in the Diagnosis of Viral and Rickettsial Diseases	2	3
Mar. 16–27	8.41	Laboratory Methods in the Diagnosis of Bacterial Diseases		
		Part 2. General Bacteriology	2	2
Mar. 16–Apr. 3	9.41	Serologic Methods in Microbiology	3	1
Mar. 30–Apr. 10	8.50	Laboratory Methods in the Diagnosis of Bacterial Diseases, Enteric Bacteriology	2	2
†	8.05	Laboratory Methods in the Diagnosis of Malaria	2	1
†	8.21	Special Training in Virus Techniques	2–4	3
†	8.42	Typing of *Corynebacterium diphtheriae*	1	2
†	8.51	Special Problems in Enteric Bacteriology	2	2
†	8.52	Phage Typing of *Salmonella typhosa*	1	2
†	8.53	Laboratory Methods in the Diagnosis of Leptospirosis	1–4	2
†	8.54	Serologic Differentiation of Streptococci	2	2
†	8.56	Bacteriophage Typing of Staphylococci	2	2

* Location of classrooms: (1) Bldg. 27, Chamblee, Georgia; (2) Bldg. 30, Chamblee, Georgia; (3) Virus Laboratory, Montgomery, Alabama.

† Courses given by special arrangement only.

COURSE IN LABORATORY METHODS IN THE DIAGNOSIS OF TUBERCULOSIS

In cooperation with the Division of Special Health Services, Public Health Service, the Microbiology Laboratories of the Communicable Disease Center, Chamblee, Georgia, will offer a course in Laboratory Methods in the Diagnosis of Tuberculosis on the following dates:

October 20—31, 1958
January 26—February 6, 1959

This course is open to all grades of employed laboratory personnel who are approved by their State Health Officers. Practical laboratory training in all phases of tuberculosis bacteriology, including preparation of culture media, microscopy, cultural procedures, diagnostic use of animals, and testing of drug sensitivity, will be included in this course. Students who attend this course will be offered a Student Extension Service for a period of one year following termination of the course.

Laboratory directors and senior laboratory staff members may also make application for this course.

No tuition or laboratory fees are charged. Reservations for this course should be made well in advance.

Application forms may be obtained from the Laboratory Branch, Communicable Disease Center, Public Health Service, P. O. Box 185, Chamblee, Georgia.

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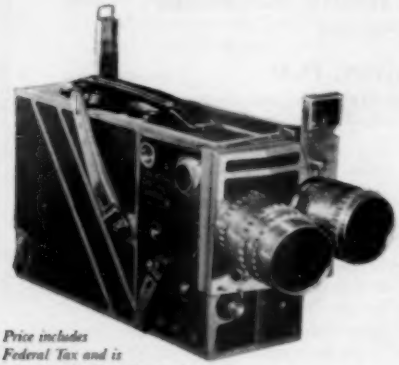

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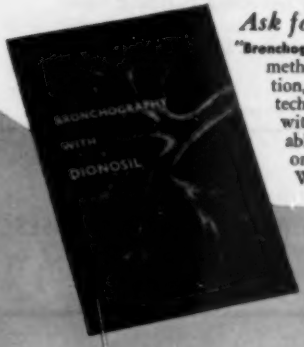

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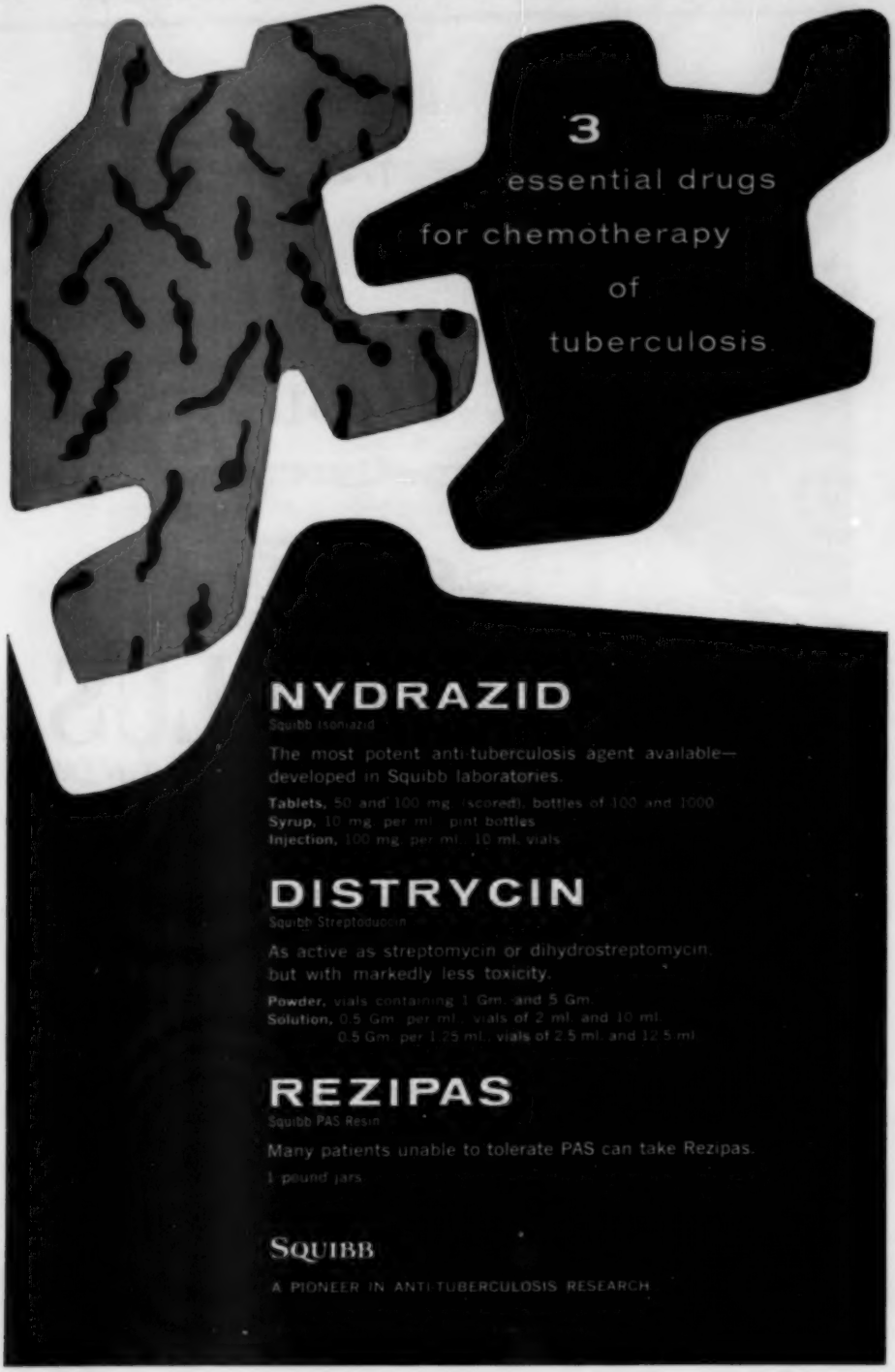
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